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# Studies on the pathogenesis of pseudorabies in domestic cats following oral exposure

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IN DOMESTIC CATS FOLLOWING ORAL EXPOSURE.

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Studies on the pathogenesis of pseudorabies  
in domestic cats following oral exposure

by

Wayne A. Hagemoser

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of  
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## INTRODUCTION

Pseudorabies (Aujeszky's Disease) has been recognized in the swine population of Europe and the United States for many years. Often the presence of this disease in a swine herd was recognized only after the death of cats, dogs, sheep, or cattle in which a "mad itch" was frequently the principal clinical sign. More recently, however, pseudorabies virus appears to have an increased virulence for swine causing abortions, stillbirths and mummified fetuses, lowered fertility, and high mortality in pigs less than 2 weeks of age. Death loss in older pigs has also been caused by pseudorabies. The incidence, as well as the virulence, of pseudorabies has increased considerably in the United States, especially in those states in which pork production is an important industry, such as Iowa, Illinois, and Indiana (Hill and Mare, 1975). An average of 5 cases of pseudorabies per year from 1969 to 1973 were diagnosed by virus isolation at the Iowa Veterinary Diagnostic Laboratory. In 1974, 28 cases; in 1975, 48 cases; in 1976, 165 cases; and in 1977, 352 cases were diagnosed by positive virus isolations. Because of the increased virulence of this herpesvirus and the increased incidence of this disease, programs for both control and eradication of pseudorabies are being considered by State and Federal agencies.

Positive diagnoses of pseudorabies are made frequently in cats and dogs on contaminated premises as well as in wild animals such as foxes (Bitsch and Munch, 1971) from the surrounding vicinity, especially during acute outbreaks. The part, if any, that these animals



contribute to the transmission of pseudorabies from farm to farm or to the persistence of the disease on an infected farm must be ascertained before adequate control and eradication programs can be effected.

The purpose of this research was to investigate the pathogenesis of an Iowa isolate of pseudorabies virus (PRV) in domestic cats under conditions which may prevail on Iowa farms. The oral route of inoculation was chosen to simulate field exposure from consumption of infected fetuses or piglets. This investigation was divided into 3 experiments each with certain objectives as follows:

Experiment I--Part A: To trace the route and time sequence of the virus from the oropharyngeal area to the brain and visceral tissues by means of virus isolation and light microscopy. To accomplish this, cats were inoculated and tissues were collected from cats killed at one day intervals.

Experiment I--Part B: To identify the exact location of the virions within the pharyngeal and nervous tissues during the course of the infection. To accomplish this, selected tissues were examined by electron microscopy.

Experiment II: To correlate histological lesions with clinical signs and to evaluate the bilateral symmetry of the nervous lesions. To facilitate this, cats were inoculated and then killed at 3 successive stages of clinical disease.

Experiment III: To evaluate the epidemiological implications of pseudorabies in cats. To accomplish this, cats were inoculated and

attempts were made daily to isolate virus from nasal and oral secretions throughout the clinical course of the disease.

## LITERATURE REVIEW

Aujeszky's disease, herein referred to as pseudorabies, has long been recognized as a consistently fatal disease of cattle, sheep, cats, dogs, and a number of other wild and domestic animals (Lamont, 1946). The disease in swine has in the past manifested itself most often as a mild, subclinical infection with high mortality in swine less than 2 to 3 weeks of age. More recently in the United States, deaths have occurred in feeder pigs and adults, and losses from abortions, stillbirths and mummified fetuses have been reported (Gordon and Luke, 1955; Kluge and Mare, 1974). The reader is referred to a paper by Baskerville, McFerran, and Dow (1973) for a thorough review of pseudorabies in swine. Among the topics discussed are epidemiology, clinical signs, pathogenesis, pathology, diagnosis, properties of pseudorabies virus, immunity, prophylaxis and treatment. No attempt will be made to cover these aspects of pseudorabies in swine in this review, rather, the literature review will concentrate on various aspects of pseudorabies in carnivores generally, and in cats more specifically. In addition two other subjects will be reviewed briefly. These are the uptake and transport of neurotropic viruses, especially herpesviruses, and the anatomical considerations of specific areas of the medulla oblongata studied in this research. These topics are considered important for an overall appreciation of the research herein presented.

## Pseudorabies in Carnivores

### Pseudorabies in cats

Clinical signs Most of the descriptions of pseudorabies in cats have been limited to the epidemiological, pathological, and histopathological aspects rather than clinical observations. Horvath and Papp (1967) reported on clinical observations in 58 cats diagnosed as having pseudorabies at the University of Veterinary Sciences, Budapest, Hungary. Anorexia, sluggishness, and depression were reported initially followed by agitation and a frequent changing of positions. Salivation, frequent meowing and retching, as if something had stuck in the throat, followed rapidly. Rectal temperatures remained normal until the terminal stages when temperatures were often subnormal. Cats scratched violently on one side of their face with the foreleg of the same side causing gross mutilation. Pruritus of the head has been reported by several workers (Metianu et al., 1971; Dow and McFerran, 1963). Horvath and Papp (1967) described an atypical form of the disease which occurred in approximately 40 percent of their cats in which agitation was not a sign and activity was generally limited to slow movements of the forelegs parallel to the body and attempts to cling to the cage or wire or other near objects. In these animals, pruritus was not present. Sabo et al. (1968) reported no signs of pruritus in 10 experimentally-infected, orally-exposed cats. Six of these, however, were killed before clinical signs had developed leaving 4 animals that had clinical signs without pruritus. These workers generally agreed that pruritus was an inconsistent clinical sign of pseudorabies in cats.

Transmission of virus      Nearly all reports of pseudorabies in cats and other carnivores implicate the feeding of swine offal in the transmission of the virus (Gore et al., 1977; Horvath and Papp, 1967; Hugoson and Rockborn, 1972). Metianu et al. (1971) were able to infect cats orally only after including bones in the food to serve as an abrasive and thus allow entrance of the virus through damaged mucous membranes. Sabo et al. (1968), however, were able to infect cats by allowing them to drink a virus-milk solution. Infection via the respiratory route, believed by Bitsch (1975, a, b, & c) to be important in the epidemiology of pseudorabies in ruminants, has not been implicated as a probable means of viral transmission in carnivores even though carnivores have been infected experimentally via aerosol spray (Ohshima et al., 1976).

Ingestion of infected rats has been suggested as a source of infection based on histories of cats and dogs which had eaten rats several days before clinical signs appeared (Cassells and Lamont, 1942). However, McFerran and Dow (1970) were unable to infect rats by close contact with virus-shedding pigs and concluded that rats were not a likely reservoir for pseudorabies virus and did not play an important role in its spread. The importance of rats in the epidemiology of pseudorabies at the present time is not completely understood.

Macroscopic lesions      Gross lesions believed to be related specifically to pseudorabies in both naturally occurring and experimental cases in cats have been limited to trauma and self-mutilation due to pruritus of the face and chin, resulting in subcutaneous hemorrhage

and edema. This is unlike reports in dogs (Gore et al., 1977) and mink (Christodoulou et al., 1970) in which pseudorabies specific lesions have been reported in the alimentary tract.

Microscopic lesions      Specific microscopic lesions reported in cats are generally limited to the brain stem and spinal cord and are essentially the same as those reported in the dog (Dow and McFerran, 1973; Knosel, 1968; Fankhauser et al., 1975). Sabo et al. (1968) described lesions in cats experimentally infected via the oropharyngeal route. Intracellular and extracellular edematous changes were noted in the tonsils. Epithelial cells of tonsillar crypts were vacuolated and sometimes completely destroyed. Intranuclear inclusion bodies were found in many relatively well-preserved epithelial cells at the margins of the larger necrotic areas. Tigrolysis and vacuolation of cytoplasm was found in some pseudounipolar cells of the trigeminal ganglion. The pathologic changes in the brain were limited to the brain stem. The meninges were infiltrated by lymphocytes. Lymphocytic perivascular cuffing was present in the gray matter of the floor of the fourth ventricle. Various degenerative changes were seen in the ganglion cells of the medial nucleus of the vestibular nerve, dorsal motor nucleus of the vagus nerve, nucleus of origin of the facial nerve and the reticular formation of the medulla. Proliferation of glial cells and lymphocytic perivascular cuffing were found in the white matter.

### Pseudorabies in dogs

Pseudorabies in a pack of hounds in which the source of virus was pig offal fed at the kennel has been reported recently by Gore et al. (1977). Lesions ranged from mild ulceration in the esophagus and stomach to severe gastritis and blood in the stomach and intestine of some. A diphtheritic appearance of the proximal portions of the small intestine was reported. Histologically, the intestinal mucosal lesions appeared less severe while the striking abnormality present involved nerve ganglia in the intermuscular layers (myenteric plexus) in which degeneration of neurons and extensive mononuclear cell infiltration were found. Similar changes were noted in the adrenal ganglion of one hound which also had a diffuse, low grade meningo-encephalitis with severe degeneration of Purkinje cells of the cerebellum.

Dow and McFerran (1963) reported on naturally occurring cases of pseudorabies in 4 dogs. Macroscopic changes were related to severe local trauma and self-mutilation at pruritic sites of the face or shoulder with swelling and congestion of the regional lymph nodes. No macroscopic lesions of the gastrointestinal tract were reported. Microscopically, specific changes were limited to the central nervous system (CNS). Degenerative changes and necrosis of ganglion cells were present in the dorsal root ganglia of the caudal cervical and cranial thoracic spinal cord segments of the dog with pruritic sites on the shoulder. In this dog, lesions were not found cranial to the nucleus cuneatus in the medulla. In 2 other dogs with pruritus of

the chin and face, lesions were most pronounced in the pons and medulla and involved especially the main sensory and spinal tract nuclei of the trigeminal nerve. Lesions ranged from central chromatolysis to necrosis of all the neurons in affected nuclei and many cells contained typical inclusions. A moderate, diffuse microglial infiltration and occasional foci of necrosis with glial and neutrophil infiltration were present in affected areas. Lesions were also present in the hypoglossal nucleus and in the dorsal motor nucleus of the vagus of one dog. Moderate mononuclear perivascular cuffing was a characteristic feature in areas where lesions were found.

Lesions of a similar type and distribution in 3 naturally-infected dogs were reported by Knosel (1968). Hugoson and Rockborn (1972) have reported an outbreak of pseudorabies in dogs caused by feeding abattoir offal. A nonpurulent meningoencephalitis, mainly localized to the medulla oblongata, was found in 2 dogs. No further details of the exact type and distribution of lesions were reported by these authors.

Fankhauser et al. (1975) also described mononuclear cell perivascular infiltration and gliosis of the brain stem of dogs with nerve cell degeneration consisting primarily of nuclear pyknosis, tigrolysis of Nissl substance and karyolysis.

#### Pseudorabies in foxes

Bitsch and Munch (1971) and Bitsch and Knox (1971) reported on pseudorabies in the red fox (Vulpes vulpes) and blue fox (Alopex lagopus) respectively in Denmark. Pig bristles were found among the stomach contents in several animals indicating infection by ingestion



of infected pork. Pruritus of the head, tail, or shoulder was present in 9 of 12 foxes in one series of cases investigated. Gross lesions in another investigation included hyperemia of the oral and pharyngeal mucosa and congestion of the stomach and small intestine. No ulcers of the alimentary tract were reported. Histologic findings were not reported by these workers, however, virus isolation studies demonstrated high titers of virus in the tonsil, medulla oblongata and pons.

Trainer and Karstad (1963) infected 2 foxes by the intramuscular and oral routes of exposure. Pruritus was present only in the animal infected intramuscularly. Microscopic lesions in both foxes were limited to hydropic degeneration, leading to necrosis of the convoluted tubules in the kidneys. Congestion, degeneration, and hemorrhages in the liver and some depletion of white pulp in the spleen were present.

#### Pseudorabies in mink

Christodoulou et al. (1970) reported an outbreak of pseudorabies in mink in Greece. Their findings indicated that the infection was food-borne, caused by the incorporation of infected swine offal into the food mixture. Clinical signs common to the majority of sick animals were anxiety and nervous excitability of variable duration and/or depression and coma. Diarrhea was present in many animals while convulsions, hypersalivation or lacrimation were seen less frequently. Abnormal movements of the tongue or scratching of the mouth area were observed in very few animals.

The most pronounced gross lesions were hemorrhagic or catarrhal enteritis and congestion of the lungs, spleen, and kidney. Microscopically, the brain was the organ most severely affected. Lymphocyte and plasma cell infiltration was present in the meninges. Necrotic foci and perivascular lymphocytic infiltration were present in the medulla oblongata.

These same workers experimentally reproduced the disease in mink by oral exposure. Congestion of the lungs, increased pericardial fluid, numerous pinpoint-size ulcers in the gastric mucosa, and congestion of the spleen were present in one animal while hemorrhagic gastroenteritis and congestion of the spleen was present in the second mink. The gross intestinal lesions in both the naturally occurring and experimentally reproduced disease in mink were remarkably similar to those described in dogs (Gore et al., 1977).

Goto et al. (1968) infected 22 of 25 mink exposed by the subcutaneous route of inoculation. Thirteen animals had clinical signs which included depression, reluctance to move, loss of consciousness and death. Nervous signs included spasms of the body, opisthotonos and extension of the legs. Gross and microscopic lesions, if found, were not reported.

Goto et al. (1971) exposed mink and ferrets by the oral, nasal, subcutaneous, and intracardiac routes. After nasal, oral, or intracardiac virus injection, virus was recovered regularly from the lungs and spinal cord. After subcutaneous infection, the virus was never isolated from the lungs, but only from the spinal cord. In no

instance was the virus found in the spleen or brain, regardless of the route of inoculation.

#### Pseudorabies in ferrets

Goto et al. (1968) described clinical signs in ferrets following subcutaneous and aerosol exposure. Anorexia was observed initially, followed by depression in the majority of animals. The animals became reluctant to move, lost consciousness, and died. Nervous signs and spasms were observed in only 3 of 16 animals. Pruritic signs were observed in several ferrets inoculated subcutaneously.

Ohshima et al. (1976) inoculated ferrets by subcutaneous, intramuscular and aerosol routes. Clinical signs included anorexia, central nervous system depression, trembling, weakness, inactivity, and hyperexcitability. Self-mutilation at inoculation sites was common as well as ataxia and posterior paralysis. Sneezing was observed when the inoculum was given by aerosol.

Macroscopic lesions included generalized enlargement of the lymphoid system. The lungs were frequently edematous in ferrets exposed by aerosol spray. The principal nervous tissue lesions in ferrets exposed by this method were located in the nucleus intercalatus, dorsal motor nucleus of the vagus nerve and in some instances in the hypoglossal nucleus. Lesions were also present in the spinal vestibular nucleus in the medulla, the pons, and the midbrain. Spinal cord lesions of these animals were predominantly in the first to fifth segments of the thoracic cord. These workers concluded that with aerosol exposure of ferrets, the distribution of the spinal cord lesions indicated that

the virus did not spread through the mucous membranes of the mouth, nostrils, or conjunctiva, but rather from the lungs or trachea to the spinal cord.

#### Pseudorabies in raccoons

There are few reports on pseudorabies in raccoons. Trainer and Karstad (1963) exposed 10 raccoons to PRV by various routes. All except one died from the exposure. Depression was the only clinical sign seen except for one animal which had been exposed by contact with other infected raccoons. Pruritus was present in this animal, as well as salivation and grinding of the teeth, accompanied by clonic convulsions and occasional tossing of the head.

Gross pathologic changes observed in the raccoons were minimal. Intramuscular exposure resulted in congestion of the turbinates, adrenal gland, kidney, spleen, and liver. Petechiation of the heart and moderate engorgement of the meningeal vessels was present in several animals. Similar but slightly more severe lesions were produced in those animals exposed by the intranasal or oral routes or by contact with infected raccoons. The only animal with signs of pruritus was one of two exposed by contact. The turbinates of this animal were inflamed and the liver, spleen, adrenal glands, and meninges appeared congested. The lungs were edematous as well as congested.

The severity of histologic findings were unrelated to the route of exposure. Lesions in 4 animals were limited to congestive and hemorrhagic changes of the heart, lungs, liver, pancreas, adrenal gland,

spleen, and kidney. Perivascular hemorrhage in the brain was found in only one of these.

Two of the 4 animals exposed intramuscularly had more pronounced lesions. These consisted of acute interstitial pneumonia; myocardial hemorrhages associated with patchy areas of myocardial fiber necrosis and fragmentation; hemorrhage and necrosis in splenic follicles; necrosis of germinal centers of lymph nodes; cortical hemorrhages in the adrenal glands and kidneys; and degenerative changes of the pancreatic ganglion cells. One animal infected by intranasal exposure had heart, lung, spleen, and adrenal gland lesions as described above, however, degenerative changes were not found in the pancreas. Glial nodules were found in the medulla oblongata of this animal. Definite inclusion bodies were not seen. However, nuclear changes consisting of margination and fragmentation of chromatin were present in glial cells.

Pseudorabies virus was recovered from a variety of tissues. The most consistent source of virus was the brain, lungs, adrenal glands, turbinates, and spleen.

The isolation of PRV from lungs and turbinates of raccoons, as well as contact transmission between raccoon pen mates suggests that the raccoon could possibly be implicated in the natural transmission of pseudorabies from farm to farm.

#### Uptake of Neurotropic Virus and Transport along Nerve Pathways

The replication of pathogenic neurotropic viruses such as rabies and pseudorabies at the site of inoculation and the pathways of these

viruses to the central nervous system are poorly understood. Several possible routes which a virus may travel have been postulated (Wright, 1953; Johnson and Mims, 1968). Among the more feasible are transport of virus outside nervous tissue proper by replication and progression by cell to cell passage through glial cells; transport of virus centrally through perineural or endoneural fluid channels; and intraaxonal transport via centripetal flow through the axoplasm. In recent years, increased support for the latter means has been gained through the research of a number of workers. Cook and Stevens (1973) inoculated herpes simplex virus into the rear footpads of mice. The infection progressed sequentially from the peripheral site of inoculation to the central nervous system, and infectious virus reached the sacrosclatic spinal ganglia in 20 to 24 hours. Immunofluorescent techniques demonstrated virus-specific antigens in both neurons and supporting cells. At the ultrastructural level, complete and incomplete virions were frequently found in ganglion and satellite cells and in axons, but were found only once in the extracellular space in sciatic nerves of mice in the acute stage of infection. In general, the work of Cook and Stevens supports the concept that herpes simplex virus can travel in neurons and their processes in the peripheral nervous system of mice. Field and Hill (1975) inoculated PRV into the ear pinna of mice and surgically removed the ears at various times after inoculation. Virus replication in the tissues of the ear and cervical dorsal root ganglia were monitored. When the inoculum was small, virus multiplication at the site of inoculation was required before infection spread up the nerve.

Larger infective doses apparently led to direct uptake of virus by the nerve. Following larger doses, virus was demonstrated in the dorsal root ganglia 17 hours after inoculation which suggested a retrograde axonal flow rate of at least 1.7 mm/hour. This rate of transport confirmed their earlier results using the sciatic nerve of mice (Field and Hill, 1974).

Recent studies in calves (McCracken et al., 1973) indicated that following subcutaneous inoculation into the shoulder area, PRV multiplied in the fascia at the inoculation site for approximately 60 hours and then appeared almost simultaneously in the entire 75 cm length of peripheral nerve, related dorsal root spinal ganglion, and spinal cord segment. Virus spread cranially and caudally along the spinal cord and was present throughout the CNS by the time of death. They established that a cell to cell progression of virus to the CNS was not possible in the time span allowed and that the vital pathway must have involved a fluid medium since the virus traveled over 75 cm in less than 72 hours. No evidence of virus transport in the perineural fluid was observed and extracellular virus was not seen ultrastructurally in either the nerve or ganglion. Evidence for the axoplasm as the pathway of the virus to the spinal ganglion and CNS was presented. Both naked and enveloped viral particles were seen in the axoplasm of nerve fibers throughout the peripheral nerve and ganglion following replication in ganglionic neurons. Virus was never observed in the extracellular spaces within the endoneurium and ganglion and was detected only in small numbers of Schwann cells and very rarely in satellite

cells. Infection of neurons was seldom accompanied by infection of surrounding satellite cells which suggested that the virus did not reach the neurons from the extracellular space of the ganglion (McCracken et al., 1973; McCracken and Dow, 1973). Support for intra-axonal movement of virus was gained when it was shown that some axoplasmic proteins are transported centrifugally at a rate exceeding that of slow components by 100 to 500 times (Lasek, 1968; McEwen and Grafstein, 1968; Ochs et al., 1969); thus some proteins can travel up to 50 cm in the axons of mammalian nerves within 24 hours. It has further been shown that a centripetal flow of some amino acids and proteins occurs in mammalian axoplasm (Watson, 1968; Kristensson and Olsson, 1971), but the rate of centripetal movement has yet to be determined. It is therefore conceivable that virions may be transported centripetally from a peripheral site to related neuronal nuclei in the spinal ganglia or cord or centripetally along nerves to cranial ganglia and the brain.

#### Neuroanatomy of Brainstem Affected by Pseudorabies

Nearly all of the more specific and consistent lesions of the brainstem of the cat produced by PRV infection in this research were located within a relatively small area of the brain. A brief review of the neuroanatomy of this area is important to understand the proposed pathogenesis of pseudorabies in the cat. The specific area is the caudodorsal medulla oblongata for several millimeters rostral and caudal to the obex. A rather thorough experimental study of the



projections of the nucleus of the tractus solitarius and the area postrema of the cat, using controlled lesions and special staining techniques to identify degenerating nerve fibers, has been published (Morest, 1967). The area postrema is a paired, highly-vascularized structure located in the floor of the fourth ventricle adjacent to the solitary nucleus laterally and the obex posteriorly. Its function is uncertain although it has been considered in relation to chemoreception and secretory activity. Morest (1960) traced axons from the neurons of the area postrema into the solitary nucleus but did not find their endings; whereas, Morest (1967) found that many terminate in the solitary nucleus, especially in its posterodorsal part. Projections of axonal and dendritic branches of neurons of the solitary nucleus into the area postrema have been described (Morest, 1960). Morest (1967) has stated, "The most significant projections of the solitary nucleus in the present study are those to the dorsal and lateral reticular formation of the medulla. Since the dorsal reticular formation has been implicated in the central regulation of respiratory, cardiovascular, and emetic activities, it is not surprising to find it in direct anatomical relationship to the region of the solitary nucleus that receives primary glossopharyngeal and vagal afferents."

Sensory branches of the glossopharyngeal and vagus nerves enter the lateral border of the medulla oblongata and along with sensory branches of the facial nerve form the distinct bundle of fibers readily identified

histologically as the tractus solitarius. Most of these fibers terminate in the nucleus solitarius. A large rostral portion of this nucleus is associated with gustatory (taste) function and receives fibers from the taste buds of the tongue and pharynx. Another group of cell bodies is identified as the dorsal sensory nucleus of the vagus and is located just medial to the tractus solitarius and dorsolateral to the dorsal motor nucleus of the vagus. A lateral portion of the nucleus is situated along the lateral border of the tractus solitarius and is referred to as the central nucleus of the tractus solitarius (Truex and Carpenter, 1969). The caudal portions of the nucleus solitarius from the right and left sides merge just dorsal and caudal to the obex to form the commissural portion of the nucleus solitarius. As fibers of the glossopharyngeal and vagus nerves enter the medulla, they either penetrate the upper one-third of the descending spinal tract and nucleus of the trigeminal nerve or arch over this structure and progress medially to eventually form a distinct tract. Efferent vagal fibers from the dorsal motor nucleus of the vagus and glossopharyngeal fibers from the nucleus ambiguus pass laterally through the mid to upper portions of the descending tract of the trigeminal nerve before they emerge through the indistinct dorsolateral sulcus on the lateral aspect of the medulla oblongata (Getty, 1975).

## EXPERIMENTATION

## Experimental Animals

Domestic cats ranging in age from 1 to 5 years were used in the study. These animals originated from homes and farms in the vicinity of Ames, Iowa, and were obtained through the Laboratory Animal Resources (LAR) section at Iowa State University. Vaccines for rabies<sup>1</sup>, feline rhinotracheitis<sup>2</sup>, feline calicivirus<sup>2</sup>, and panleukopenia<sup>3</sup> were administered upon entering LAR facilities where cats were caged individually prior to assignment to the present project. All cats were examined serologically for pseudorabies antibodies and virologically for feline rhinotracheitis and feline calicivirus before being assigned to experimental groups. Oral and nasal secretions, collected on swabs from each cat were inoculated onto Crandell feline kidney (CFK) cells with negative results, and serum neutralization (SN) titers for pseudorabies virus were <1:2 (Hill et al., 1977). Cats were caged individually throughout the experiments.

## Virus Inoculum

The virus inoculum was originally isolated from a pig in a naturally-occurring outbreak of pseudorabies in swine in Northwest Iowa. The virus

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<sup>1</sup> Endurall-R	Norden Laboratories	Lincoln, Nebraska.
<sup>2</sup> Felomune CVR	Norden Laboratories	Lincoln, Nebraska.
<sup>3</sup> Felocine	Norden Laboratories	Lincoln, Nebraska.

was propagated on pig kidney cells (PK15)<sup>1</sup> and a pool of virus from the 7th passage was frozen at -70 C. Aliquots were subsequently tested over the range of time of the experiment. The titer remained stable in a range of 1 to  $2.3 \times 10^7$  plaque forming units (pfu) per ml.

#### Experiment I--Part A

##### Materials and methods

Animals Thirty-three cats were used, 4 uninoculated controls and 29 inoculated animals. Each inoculated cat was given 0.1 ml of virus stock suspended in 0.9 ml phosphate buffered saline (PBS) orally by dose

Table 1. Experiment I--Part A: Assignment of cats to experimental groups.

Group	Cat identification numbers							
Control	11	12	35	37				
Killed 1 DPI <sup>a</sup>	13	16	36	38				
Killed 2 DPI	8	10	25	32	43	45	48	
Killed 3 DPI	17	23	24	27	33	40	52	60
Killed 4 DPI	7	14	30	34	42	59		
Killed 5 DPI	9	19	28	31				

<sup>a</sup>Days post inoculation.

<sup>1</sup>American Type Culture Collection, Cell Repository, Washington, D.C.

syringe. Animals were killed by electrocution at 1, 2, 3, 4, and 5 days post inoculation (DPI). Postmortem examinations were performed immediately after death.

Virus isolation      Specimens for virus isolation from all 33 cats were gathered and stored at -70 C until processed. The following specimens were collected for virus isolation: cerebral hemisphere; cerebellum; mid-brain; medulla; pons; olfactory bulb; composite of thalamus and hypothalamus; cervical, thoracic, and lumbosacral spinal cord segments; medial retropharyngeal lymph node (ln); composite of pharyngeal mucosa and tonsil; nasal turbinates; mesenteric ln; adrenal gland; mandibular salivary gland; kidney; liver; spleen; stomach; composite of jejunum and ileum; colon; heart; lung; and colonic contents. The brain was hemisected longitudinally. One-half was used for virus isolation studies and the other half for histological procedures. The spinal cord was divided into cervical, thoracic, and lumbosacral segments. Each segment was again divided into 4 pieces of equal length. The proximal and 3rd pieces were processed for histological evaluation and virus isolation studies were performed on the 2nd and distal pieces of each segment. All virus isolations were performed by the Iowa Veterinary Diagnostic Laboratory. A 10 percent suspension of these tissues in Earle's balanced salt solution (EBSS) containing 0.11%  $\text{NaHCO}_3$ , 1,000  $\mu\text{g/ml}$  streptomycin, 1,000 units/ml penicillin and 5  $\mu\text{g/ml}$  Fungizone<sup>®</sup> <sup>1</sup> was inoculated onto CFK and PK15 cells.

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<sup>1</sup>E. R. Squibb and Sons, Princeton, New Jersey.

Specimens were incubated under 5 percent CO<sub>2</sub> at 37 C for 7 days. Cultures were examined daily for cytopathic effect (CPE). Cultures which failed to show CPE after 7 days were repassed twice before being considered negative. A typical herpes CPE on either feline or porcine kidney cells was considered positive. Positive isolations were confirmed by the fluorescein labeled antibody staining technique.

Light microscopy Tissue specimens for histopathological examination from all 33 cats were fixed in 10 percent neutral buffered formalin, processed by routine paraffin techniques and sectioned at 6 µm. Besides the tissues collected for virus isolation, the following additional tissues were taken: trigeminal (semilunar) ganglion; pituitary gland; thyroid gland; tongue; esophagus; and pancreas. The medulla oblongata was sectioned transversely in its entirety. Two sections were taken from each of 10 equally spaced areas of the medulla. These sections were stained with hematoxylin and eosin (H&E) according to the routine Harris method and luxol fast blue-cresyl echt violet (LFB-CV) according to the Kluver-Barrera method for myelin and nerve cells (U.S. Armed Forces, 1968). All other tissues were stained with H&E only.

## Results

Macroscopic lesions Macroscopic lesions believed to be specific for pseudorabies were not present in any cats. Nonspecific consolidation of the apical and cardiac lobes of the lung and varying amounts of light and dark pink mottling of the entire lung parenchyma were the

most frequently found lesions. Notably, no gross lesions were found in the tonsils, nasopharynx, alimentary canal or nervous system.

Microscopic lesions of non-nervous tissue

Lung Thirty of 33 animals had some degree of pulmonary change. Lesions consisted mainly of a mild to moderate amount of atelectasis and a slight, generalized increase in the thickness of the alveolar walls primarily due to increased numbers of mononuclear septal cells. Alveoli, bronchioles and bronchi were generally free of exudate except for focal intra-alveolar hemorrhage in some lungs. Mild to moderate bronchopneumonia was present in a 3, 4, and 5-DPI animal. No correlation was found between the frequency and severity of the lung lesions and the experimental groups from which these animals came.

Turbinates A mild to moderate diffuse infiltration of lymphocytes and neutrophils (PMN's) was present in the submucosa of many turbinates. Occasionally, dense accumulations of PMN's, fibrin, and mucus were present on the mucosal surface. Small ulcerated foci moderately infiltrated with PMN's were present in the mucosa of several turbinates. Varying degrees of turbinate inflammation were present in 27 of 33 cats. Control as well as inoculated animals appeared equally affected.

Tonsils A mild to moderate infiltration of lymphocytes and plasma cells into the basilar layers of the crypt epithelia was present in the 4 control animals (Figures 1 and 2) and in 3 of the 4 cats killed 1 DPI. This finding was considered normal for this group of animals. Ulceration of the crypt epithelium with moderate to marked

Figure 1. Tonsil, Cat 12, Control, Experiment I--Part A. Infiltration of lymphocytes and plasma cells into the basilar area of the tonsillar crypt epithelium. Note epithelium is not ulcerated. H & E. x132

Figure 2. Tonsil, Cat 37, Control, Experiment I--Part A. Dense concentrations of lymphocytes within the tonsillar crypt epithelium. H & E. x165



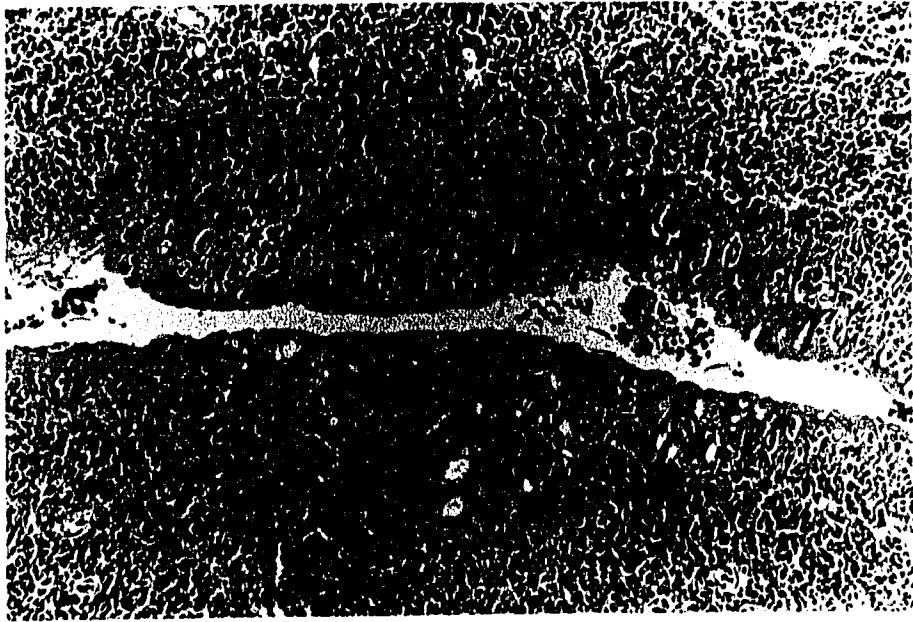


Figure 3. Tonsil, Cat 38, 1 DPI, Experiment I--Part A. PMN's, mononuclear cells and cellular debris in tonsillar crypt lumen. Note multiple foci of crypt epithelial ulceration. H & E. x132

Figure 4. Tonsil, Cat 38, 1 DPI, Experiment I--Part A. Greater magnification of area from Figure 3. Note intraepithelial accumulation of several PMN's (arrow). H & E. x330

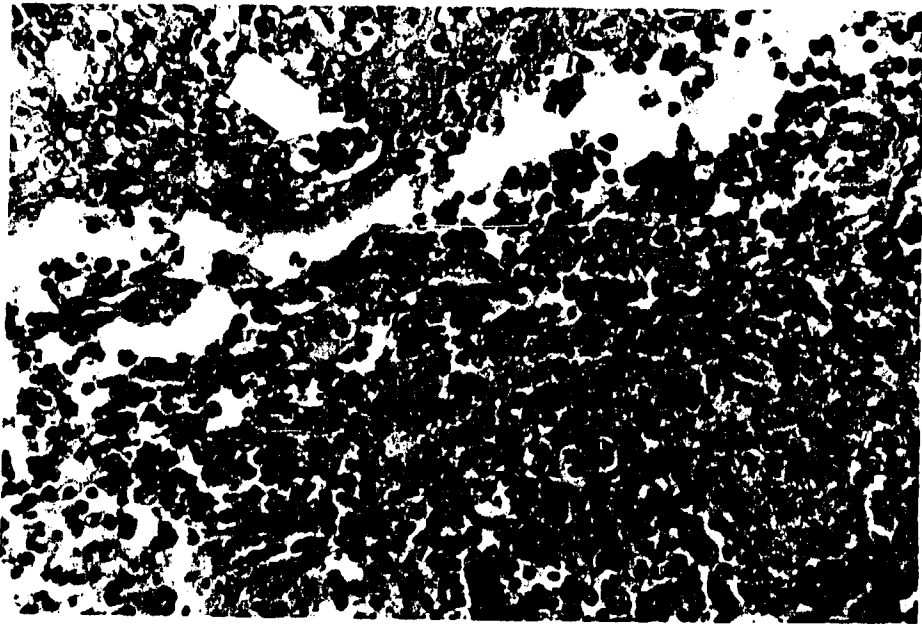
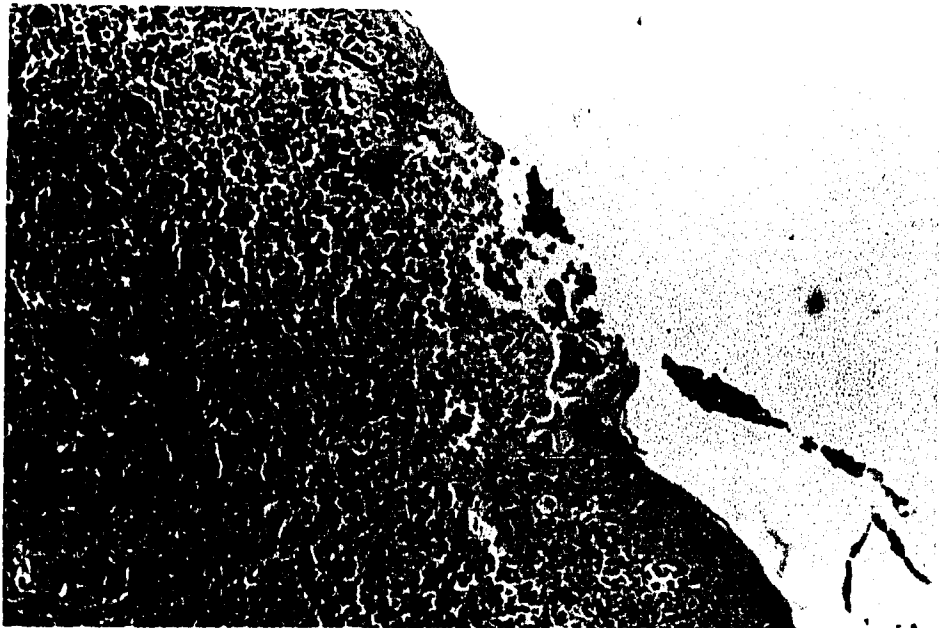
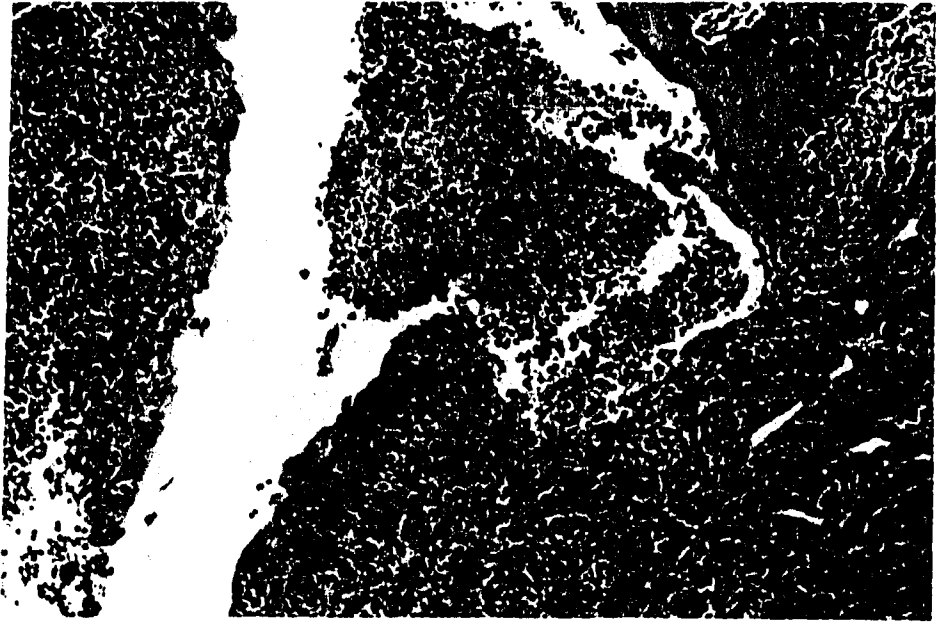


Figure 5. Tonsil, Cat 25, 2 DPI, Experiment I--Part A. PMN's and mononuclear cells present in tonsillar crypt. Focal areas of ulceration of crypt epithelium. H & E. x132

Figure 6. Tonsil, Cat 30, 4 DPI, Experiment I--Part A. Focal ulceration of crypt epithelium. Note mild hydropic changes in some epithelial cells adjacent to ulcer. H & E. x132



infiltration of PMN's into the subepithelial and perifollicular interstitial tissues, especially in the ulcerated areas, characterized the 4th animal killed 1 DPI (Figures 3 and 4) and many of the cats killed 2 DPI and later (Figures 5 and 6). Varying numbers of PMN's and amounts of cellular debris were present in the crypts and vacuolation of epithelial cells adjacent to ulcerated foci was often present. Some degree of focal to diffuse crypt ulceration was present in 13 of 25 cats killed 2 DPI or later, and lesions similar to those described above were found in all except 2 animals killed later than 1 DPI.

Microscopic lesions of nervous tissues (Table 2)

Brain

Control      No microscopic lesions were present in any of the 4 control cats.

Cats killed 1 DPI      No microscopic lesions were present in any of the 4 cats killed 1 DPI.

Cats killed 2 DPI      Microscopic lesions were present in 3 of 7 cats killed 2 DPI. Slight lymphocytic cuffing of a single vessel ventral to the tractus solitarius (T. sol.) in the caudal medulla was the only lesion present in one animal (43)<sup>1</sup>. Mild lymphocytic cuffing of several vessels and very small microglial nodules of 10 to 12 cells in and around the T. sol. were present in another cat (45).

In the 3rd animal (25), lesions were somewhat atypical in distribution from cats killed 2 DPI and later. Mild lymphocytic perivascular

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<sup>1</sup>Experimental number of cat referred to in text.

Table 2. Experiment I--Part A: Microscopic lesions of nervous tissue.

Cat Number	Days Post Inoculation																								
	2								3								4				5				
	8	10	25	32	43	45	48	17	23	24	27	33	40	52	60	7	14	30	34	42	59	9	19	28	31
Cerebral hemisphere	- <sup>a</sup>	-	+ <sup>b</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Cerebellum	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Medulla	-	-	++ <sup>c</sup>	-	+	+	-	+	++	+	++	++	++	++	++	++	++	+++ <sup>d</sup>	++	++	++	+++	+++	+++	++
Pons	-	-	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	-	+	+	-	+	++	++	-
Midbrain	-	-	N <sup>e</sup>	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-
Thalamus-hypothalamus	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	++	-	-	-
Olfactory bulb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cervical cord	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-
Thoracic cord	-	-	++	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
Lumbosacral cord	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	+	-	-	-
Trigeminal ganglion	N	-	-	N	-	-	-	-	-	N	+	+	++	-	+	-	-	-	+	-	+	N	N	-	-

<sup>a</sup> No lesion present.

<sup>b</sup> Mild lesions.

<sup>c</sup> Moderate lesions.

<sup>d</sup> Marked lesions.

<sup>e</sup> Tissue was not examined.

cuffing of several cerebral cortical vessels was present and several vessels were dilated and the lumina contained accumulations of lymphocytes and PMN's. Scattered microglial nodules and mild lymphocytic cuffing of scattered vessels within the central white matter of the cerebellum as well as mild dilation and cuffing of leptomeningeal vessels were present (Figures 7 and 8). Moderate lymphocytic cuffing of major vessels and moderate microgliosis in the area of the nucleus ambiguus were found. Mild gliosis along the course of the dorsal branches of the 9th (glossopharyngeal) and the 10th (vagus) cranial nerves, as well as mild cuffing of several vessels at the point where these nerves emerge from the brain stem, were found. Lesions within the nucleus or tractus solitarius were very mild. Slight lymphocytic cuffing of scattered parenchymal vessels was present in the pons, midbrain, thalamus, and hypothalamus. Lesions were present in all segments of the spinal cord. Moderate lymphocytic vascular cuffing in the lateral funniculus, as well as mild to moderate microgliosis and slight cuffing of the vessels in the central gray matter, were present in the cervical cord. Marked cuffing of a vessel in the ventral sulcus and in the lateral area of the gray H were present in the thoracic cord (Figure 9). Moderate to marked lymphocytic vascular cuffing and multifocal gliosis were present in the lumbosacral cord segment.

Cats killed 3 DPI      Lesions were present in the medulla oblongata in 8 of 8 cats killed 3 DPI. Six of the 8 cats also had lesions in the pons. In addition, single animals had lesions in the



Figure 7. Cerebellum, Cat 25, 2 DPI, Experiment I--Part A. Microglial nodule and mild lymphocytic vascular cuffing in the central white tracts of the cerebellum. H & E. x33

Figure 8. Cerebellum, Cat 25, 2 DPI, Experiment I--Part A. Greater magnification of microglial nodule and adjacent lymphocytic perivascular cuffing from Figure 7. H & E. x165

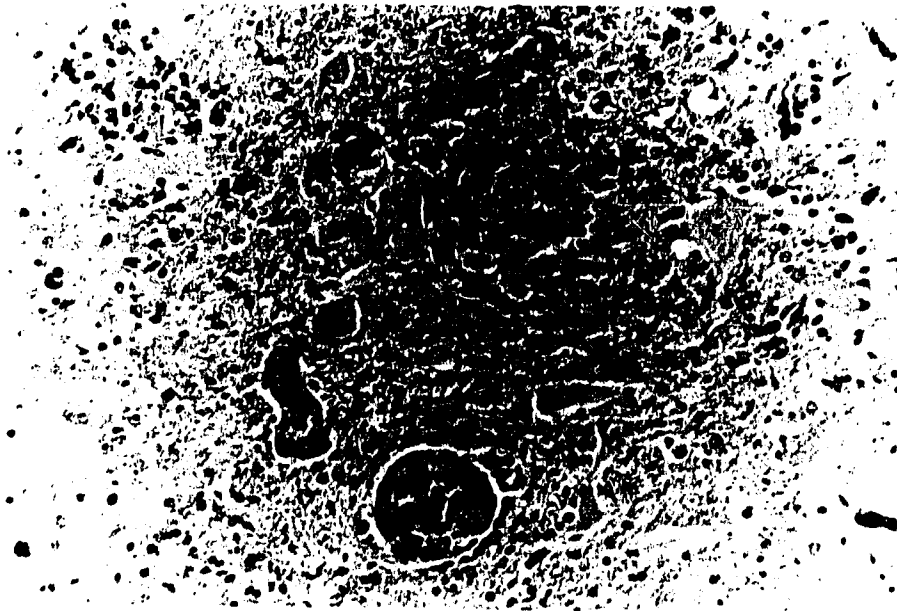
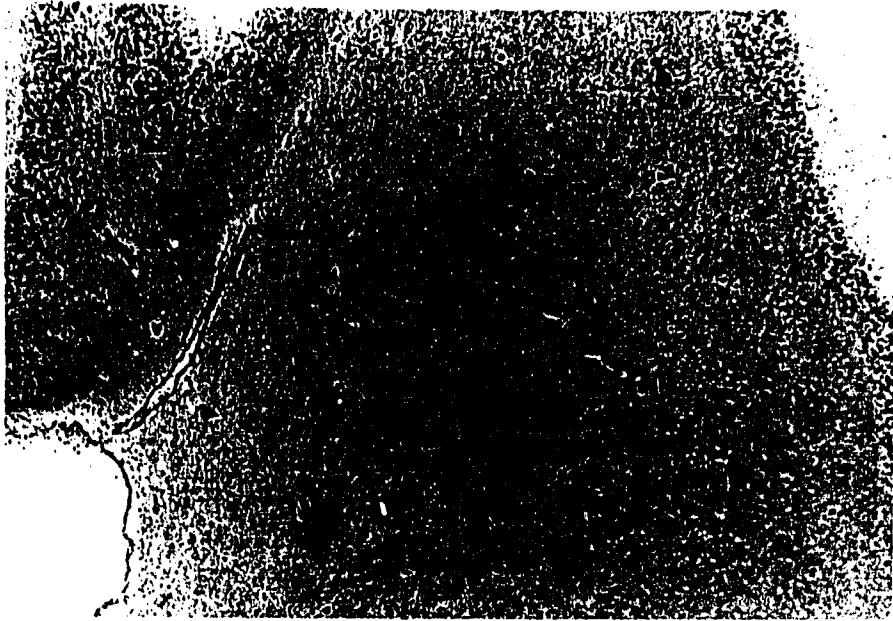
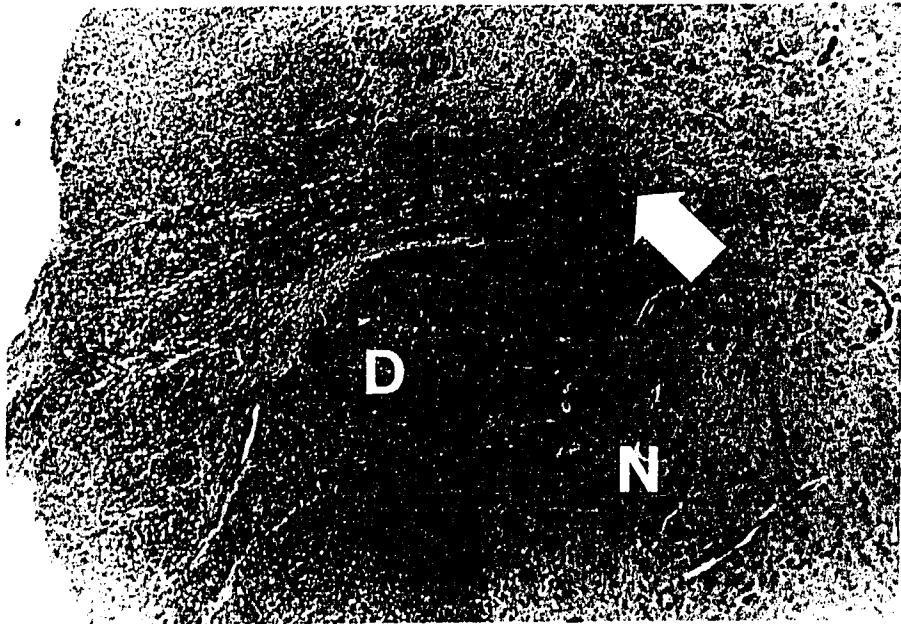
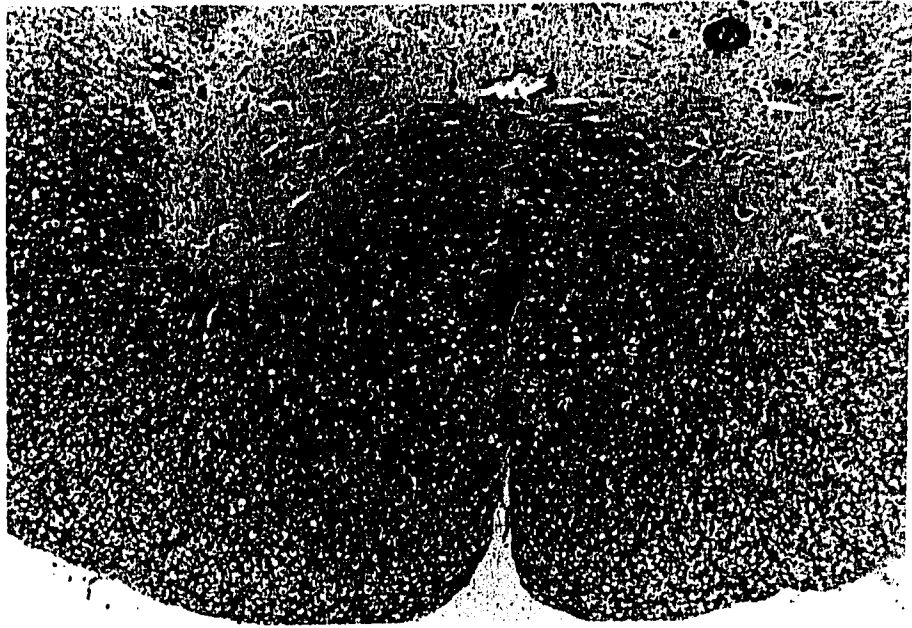


Figure 9. Thoracic spinal cord, Cat 25, 2 DPI, Experiment I--Part A. Mild microgliosis and moderate to marked lymphocytic vascular cuffing in the right dorsolateral portion of the gray H and in the ventral sulcus. x33

Figure 10. Medulla, Cat 23, 3 DPI, Experiment I--Part A. Microglial nodule (arrow) along the pathway of the dorsal branches of the 9th and 10th cranial nerves as these fibers arch over the dorsal aspect of the descending spinal tract (D) and nucleus (N) of the trigeminal nerve. x33



midbrain (40), cervical cord (33), or thoracic cord (17). Lesions in the medulla in this group of cats varied somewhat in intensity but were quite similar in anatomical location and in the nature of the inflammatory response. Small microglial nodules were frequently present along the pathway of the dorsal branches of the 9th and 10th cranial nerves as these fibers arched over or passed through the dorsal portion of the descending tract and nucleus of the trigeminal nerve (Figure 10). An intense focal gliosis was present in some cats at the point of emergence of the above nerves from the lateral margins of the medulla. Mild to moderate focal and diffuse microgliosis and moderate lymphocytic perivascular cuffing were present in the tractus and nucleus solitarius and area postrema (Figures 11 and 12). Foci of gliosis and cuffing of vessels were also noted in the commissural portion of the nucleus solitarius (N. sol.) (Figures 13 and 14) and in the region of the accessory cuneate and cuneate nuclei. Lesions were present in the pons of 6 of 8 cats killed 3 DPI. These lesions were similar in nature, though generally less severe and more randomly scattered throughout the parenchyma, than those in the medulla. Some tendency for the gliosis to occur along the pathways of the 7th and 9th cranial nerves as they coursed through the parenchyma and emerged at the lateral surface was present. Mild lymphocytic cuffing of a vessel dorsolateral to the cerebral peduncle and mild focal gliosis at the point of entrance of the dorsal spinal nerve root were found in the midbrain and cervical cord respectively of a single animal.

Figure 11. Medulla, Cat 23, 3 DPI, Experiment I--Part A. Focal and diffuse microgliosis and mild lymphocytic perivascular cuffing in the tractus and nucleus solitarius (arrow) and area postrema (A). x33

Figure 12. Medulla, Cat 23, 3 DPI, Experiment I--Part A. Greater magnification of tractus solitarius from Figure 12. Note focal microgliosis within and around the tract. xl65

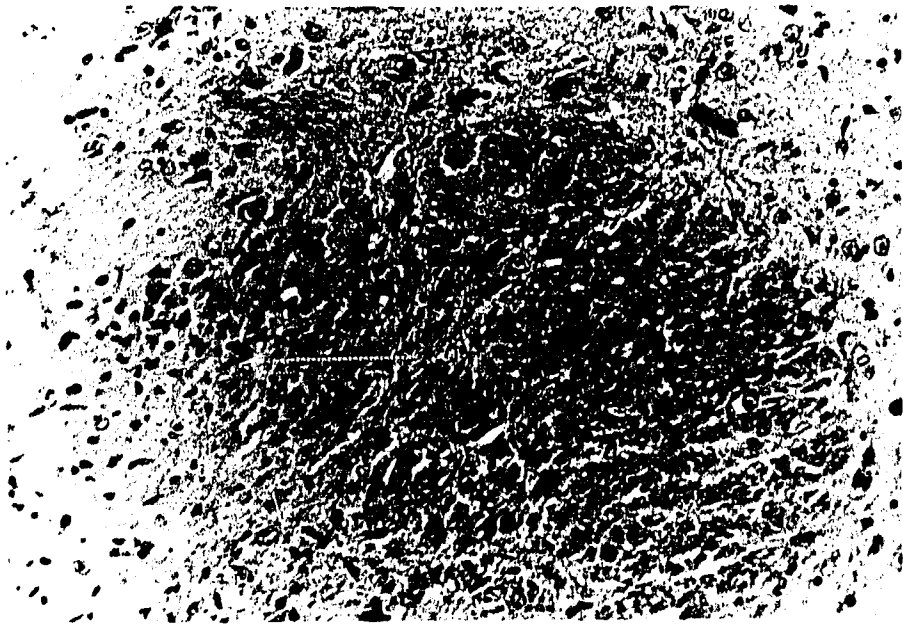
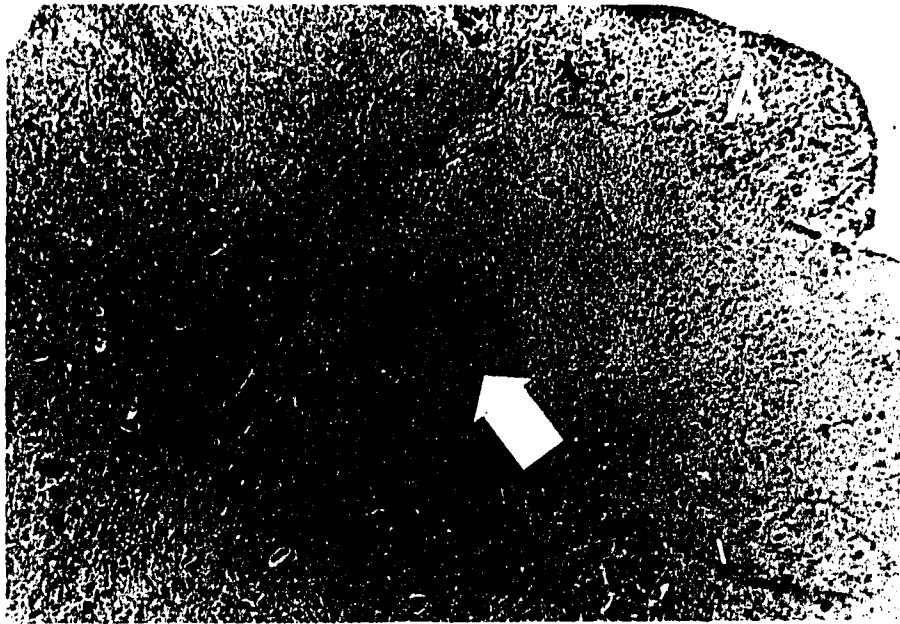
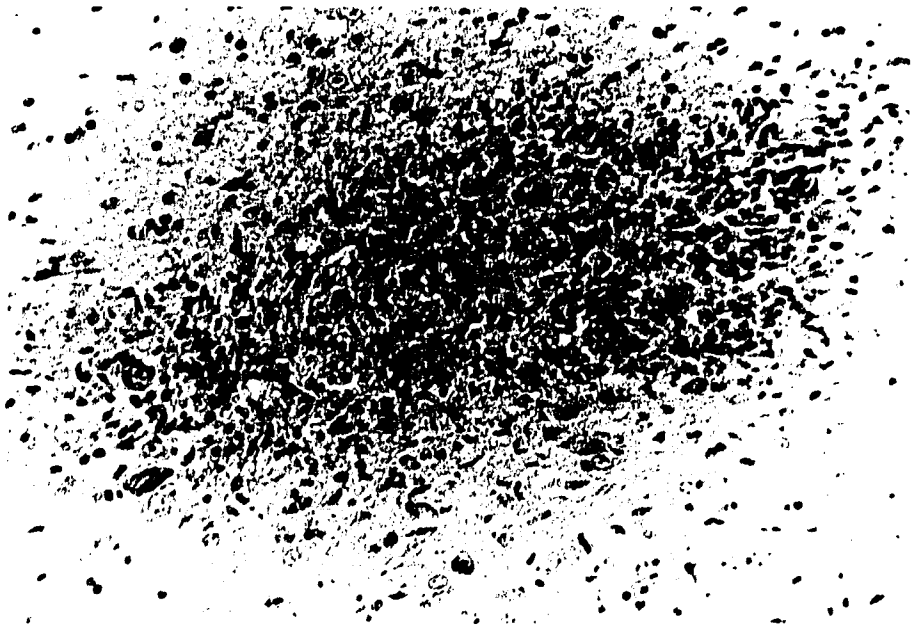
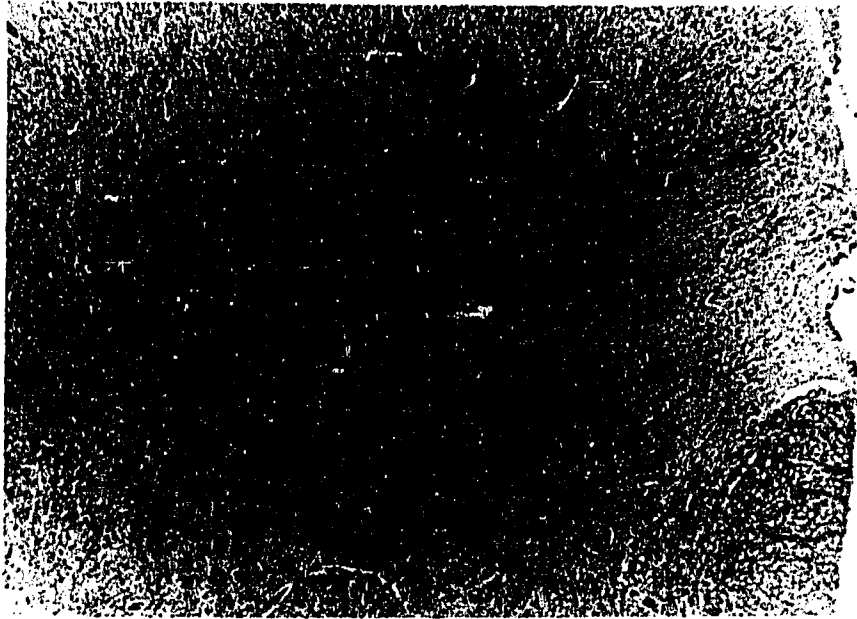


Figure 13. Medulla, Cat 23, 3 DPI, Experiment I--Part A. Marked focal microgliosis medial to the tractus solitarius and in the commissural portion of the nucleus solitarius. H & E. x33

Figure 14. Medulla, Cat 23, 3 DPI, Experiment I--Part A. Greater magnification of microglial nodule from Figure 13. H & E. x165



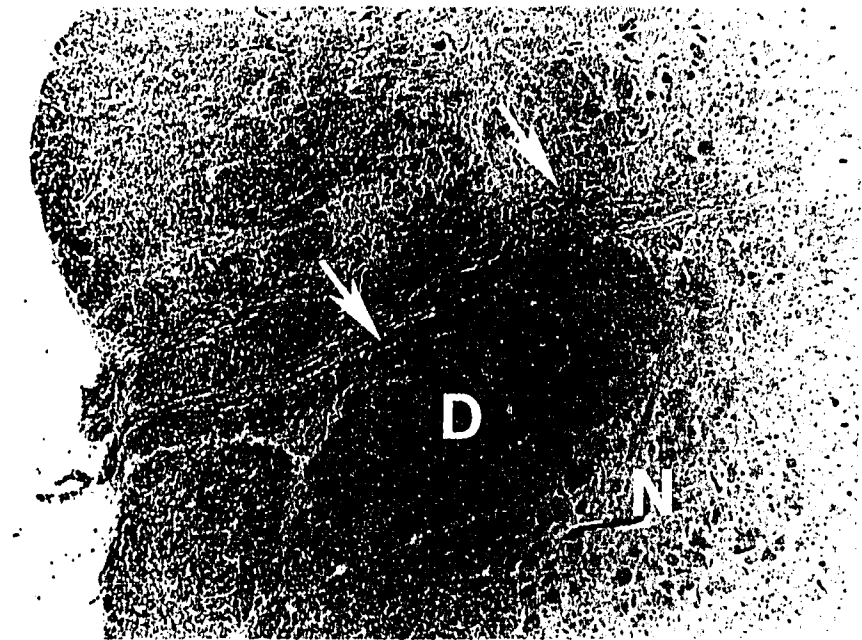


Cats killed 4 DPI      The lesions found in this group were similar in nature and distribution to those found in the 3-DPI cats although they were evaluated subjectively to be somewhat more severe. Of the 6 cats examined 4 DPI, all 6 had lesions of the medulla, 3 had pontine lesions and 2 had lesions of the midbrain with 1 each having lesions in the cervical or thoracic cord.

Cats killed 5 DPI      Microgliosis and vascular cuffing of the medulla were moderate in 3 and moderately severe in the 4th cat killed 5 DPI and mild pontine lesions were present in 3 of the 4 animals. No lesions were found in the midbrain. However, moderately severe lesions were present in the thalamo-hypothalamic area in one cat which also had thoracic and lumbosacral cord lesions. One other cat had cervical cord lesions. Focal gliosis along the course of the 9th and 10th cranial nerves through the medulla were present in several cats (Figure 15). The extent and severity of medullary and pontine lesions in 3 of the 4 animals were found to be at least as marked as any in the study.

Trigeminal ganglion      Trigeminal ganglia from 6 cats were either lost in processing or found inadequate for proper evaluation. No lesions were present in control animals. A lesion was found in one specimen of 8 examined from inoculated cats killed before 3 DPI. Lesions were present in 4 of 7 specimens from cats killed 3 DPI. In one of these (40), 2 foci of marked gliosis containing several PMN's

Figure 15. Medulla, Cat 28, 5 DPI, Experiment I--Part A. Several foci of microgliosis (arrows) along the pathway of the 9th and 10th cranial nerves as they pass over the descending spinal tract (D) and nucleus (N) of the trigeminal nerve. H & E.  
x33



were present. Several focal areas of microgliosis were found in which neuronophagia of ganglion cells was evident (Figures 16 and 17). In 2 of 8 ganglia examined from cats killed 4 DPI and later, somewhat milder lesions including several small interstitial foci of gliosis were found.

Virus isolation (Tables 3 and 4)

Control No positive virus isolations were made from any of the 4 control cats.

Cats killed 1 DPI Virus was isolated from the pharyngeal mucosa and tonsil in 4 of 4 cats. Turbinates yielded virus in 2 animals and in 1 of these, virus was also found in the medulla, pons, midbrain and cervical spinal cord.

Cats killed 2 DPI The pharyngeal mucosa and tonsil of 6 of 7 cats yielded virus. Virus was isolated from the turbinates of the 1 cat in which no virus was isolated from the pharyngeal area. The salivary glands and cervical cord also yielded virus in a single cat.

Cats killed 3 DPI The pharyngeal mucosa and tonsil yielded virus in 7 of 8 cats. Lungs and adrenal glands yielded virus in the animal from which no virus was found in the pharyngeal tissues. Virus was found in 1 other lung and adrenal gland. Virus was isolated from the turbinates of 3 animals. Nervous tissue was more consistently positive for virus than in the preceding groups. Virus was isolated from the medulla of 4, midbrain of 3, pons of 2, cerebellum of 2, thalamus-hypothalamus of 1, cervical cord of 3, and thoracic cord of 1 cat. Virus was not isolated from the nervous tissue of 3 cats.

Figure 16. Trigeminal ganglion, Cat 40, 3 DPI, Experiment I--Part A.  
Interstitial microglial nodule. H & E. x83

Figure 17. Trigeminal ganglion, Cat 40, 3 DPI, Experiment I--Part A.  
Greater magnification of microglial nodule from Figure 16.  
Note satellitosis and neuronophagia of several ganglion  
cell bodies (arrows) within the nodule. H & E. x330

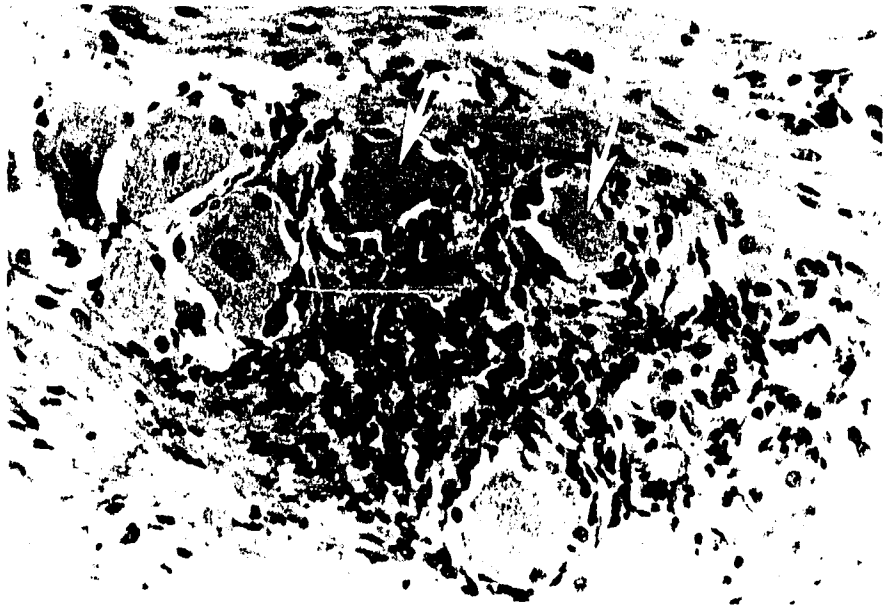
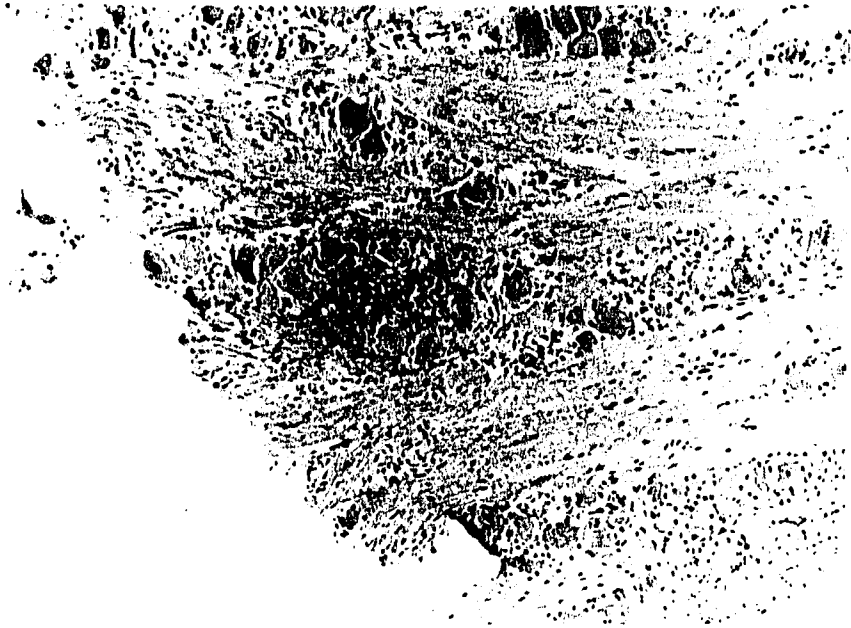


Table 3. Experiment I--Part A: Results of 825 PRV isolation attempts<sup>a</sup> on tissues from control and inoculated cats.

	Control				Days Post Inoculation										
	11	12	35	37	13	16	36	38	8	10	25	32	43	45	48
Cat Number															
Cerebral hemisphere															
Cerebellum															
Medulla								++ <sup>b</sup>							
Pons								++							
Midbrain								++							
Thalamus-hypothalamus															
Cervical cord								0+ <sup>c</sup>			+0 <sup>d</sup>				
Thoracic cord															
Medial retro-pharyngeal ln.															
Pharyngeal mucosa and tonsil															
Turbinates															
Mesenteric ln.															
Adrenal gland															
Salivary gland															
Lung															

Olfactory bulb, lumbosacral cord, kidney, liver, spleen, jejunum-ileum, stomach, colon, heart, colon contents consistently negative.

<sup>a</sup> Virus isolations done simultaneously on porcine and feline kidney cells.

<sup>b</sup> Positive isolations on porcine and feline cells.

<sup>c</sup> Positive isolation on feline cells only.

<sup>d</sup> Positive isolation on porcine cells only.





Table 4. Experiment I--Part A: Summary of results of 725 PRV isolation attempts on tissues from inoculated cats.

	Days post inoculation				
	1	2	3	4	5
Cerebral hemisphere	0/4 <sup>a</sup>	0/7	0/8	1/6	1/4
Cerebellum	0/4	0/7	2/8	1/6	1/4
Medulla	1/4	0/7	4/8	4/6	3/4
Pons	1/4	0/7	2/8	2/6	3/4
Midbrain	1/4	0/7	2/8	3/6	3/4
Thalamus-hypothalamus	0/4	0/7	1/8	1/6	3/4
Cervical cord	1/4	1/7	3/8	1/6	2/4
Thoracic cord	0/4	0/7	1/8	1/6	2/4
Medial retropharyngeal ln.	0/4	0/7	0/8	0/6	2/4
Pharyngeal mucosa & tonsil	4/4	6/7	7/8	5/6	3/4
Turbinates	2/4	1/7	3/8	2/6	1/4
Mesenteric ln.	0/4	0/7	0/8	1/6	0/4
Adrenal gland	0/4	0/7	2/8	0/6	0/4
Salivary gland	0/4	1/7	0/8	1/6	0/4
Lung	0/4	0/7	2/8	1/6	0/4

Olfactory bulb, lumbosacral cord, kidney, liver, spleen, jejunum-ileum, stomach, colon, heart, colon contents were consistently virus negative.

<sup>a</sup> Number positive isolations/number isolation attempts.

Cats killed 4 DPI      Virus was not found in any of the tissues of 1 of these animals. The pharyngeal tissues yielded virus in all of the remaining 6 cats. Virus was found in the salivary gland, mesenteric lymph node and turbinate of 1 cat and in the lungs and turbinate of another. Virus was isolated from the medulla of 4, midbrain of 3, pons of 2, cerebellum of 1, thalamus-hypothalamus of 1, cervical cord of 1, thoracic cord of 1, and cerebral hemisphere of 1 cat.

Cats killed 5 DPI      All tissues from 1 cat in this group were negative for virus. The pharyngeal mucosa and tonsil yielded virus in the other 3 cats. The turbinates yielded virus in 1 cat and the medial retropharyngeal lymph node yielded virus in 2 cats. The medulla, midbrain, pons and thalamus-hypothalamus all yielded virus in 3 cats. In addition, the cervical and thoracic cord, cerebellum and cerebral hemisphere yielded virus in 1 and the cervical and thoracic cord yielded virus in another.

In summary, the pharyngeal mucosa-tonsil composite was the non-nervous tissue which most consistently yielded virus. In descending order of frequency, the medulla, midbrain, and pons most often yielded virus.

#### Experiment I--Part B

##### Materials and methods

The presence of virus within certain tissues of pseudorabies-infected cats was confirmed with virus isolation studies correlated with the histopathologic findings. An additional effort was made to locate the virions

within the tissues using ultrastructural techniques. To accomplish this, 3 cats, Numbers 81, 82, and 83 were inoculated orally in a manner similar to those in Experiment I—Part A. Cat 84 served as an uninoculated control. The inoculated cats were killed 4, 5, and 7 DPI. All 4 cats were killed by perfusion. The perfusate contained the following in 0.1 M Sorensen's phosphate buffer:

2.5% glutaraldehyde  
2.0% paraformaldehyde  
9.5% sucrose  
final pH = 7.05

All were anesthetized with Ketaset<sup>®</sup> <sup>1</sup>. The descending aorta was exposed by a ventral midline incision in the abdomen and a small polyethylene catheter was inserted into the aorta and advanced cranially to near the diaphragm. The right chest wall was incised and the right atrium was opened to allow escape of perfusate. Approximately 1 liter of fixative was perfused in 30 minutes. Postmortem examination on each cat was performed immediately after perfusion. Tonsil, trigeminal ganglion, proximal ganglion of the glossopharyngeal nerve, and the caudal medulla in the vicinity of the area postrema and the tractus and nucleus solitarius were collected. The brain and head region of Cats 81 and 83 failed to perfuse adequately and fixation of these tissues was poor. Tissues from the poorly perfused cats were cut into small pieces and immersed in the perfusate at 4 C for 24 hours. Fixed tissues from all four cats were cut into 1 mm<sup>3</sup> pieces and processed for electron microscopy. Specimens were postfixed in 1 percent osmium tetroxide and

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<sup>1</sup>Bristol-Myers Co., Syracuse, New York.

dehydrated by passage through several changes of acidified 2,2-dimethoxypropane (DMP). The next to last solution contained 1:1 proportions of DMP and epon mixture and the last solution contained only the epon mixture. Tissues were then embedded in epon. The epon mixture<sup>1</sup> was prepared as follows:

Epoxy resin	Epon 812	16.82 grams
Dodecenyl succinic anhydride	DDSA	8.06 grams
Nadic methylanhydride	NMA	8.70 grams
Tri (Dimethyl Amino Methyl) Phenol	DMP-30	0.30 grams

Sections 3  $\mu$ m thick were cut using glass knives and a LKB Type 4801A Ultratome<sup>2</sup>. Thick sections were mounted on glass slides at 60 C, stained with toluidine blue for 15 to 20 seconds and washed with tap water. Cover slips were mounted with Coverbond<sup>3</sup>. After selection of specific areas to be examined, blocks were trimmed to give a face approximately 0.5 mm square. Ultrathin sections were cut at approximately 500  $\text{\AA}$  with an LKB 8800 Ultratome III<sup>2</sup> using diamond knives. The sections were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined with a Hitachi HS-9 electron microscope<sup>4</sup> at 75 Kv.

### Results

Previous unpublished findings have shown that PRV virions can be identified in tissues even after advanced autolysis has taken place. Consequently, the failure of the head of several animals to fix properly following perfusion was not considered detrimental to the experiment.

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<sup>1</sup>Ladd Research Industries, Inc., Burlington, Vermont.

<sup>2</sup>LKB Instruments, Inc., 12221 Parklawn Drive, Rockville, Maryland.

<sup>3</sup>American Hospital Supply Corp., McGraw Park, Illinois.

<sup>4</sup>Hitachi, Ltd., Tokyo, Japan.

Lymphocytic cuffing of vessels within the medulla and occasional neurons with degenerating nuclei as well as increased microgliosis of the neuropil could be seen in plastic embedded thick sections from all inoculated animals. Virions, however, were demonstrable only occasionally within the nucleus of scattered neurons in ultrathin sections of Cat 83 killed 7 DPI (Figures 18 and 19). Foci of inflammation were not found in thick sections of ganglia of any cats, nor did electron microscopy reveal virions within these tissues.

Foci of degeneration of tonsillar crypt epithelium were present in some of the thick sections from all three cats. However, no epithelial cells were found in which the nucleus was undergoing degenerative changes as evidenced by margination of chromatin or inclusion-like materials within the nucleus. No foci of ulceration of crypt epithelium were found in the thick sections. Neutrophils and intraepithelial lymphocytes were present in foci of crypt epithelial degeneration, but virions within these tissues could not be demonstrated in ultrathin sections.

## Experiment II

### Materials and methods

Animals Twelve cats were assigned randomly to one of 3 experimental groups (Table 5). Group 1 cats were killed at the earliest sign of clinical disease. Group 2 cats were killed after more advanced clinical signs developed, and Group 3 cats were killed in extremis or allowed to progress to death. These cats were inoculated orally in

Figure 18. Dorsocaudal medulla, Cat 83, 7 DPI, Experiment I--Part B. Margination of chromatin within the nucleus (N) of a neuron. Note granular appearing viral matrix in the center of the nucleus containing viral particles within the karyoplasm. Uranyl acetate and lead citrate. x19,250

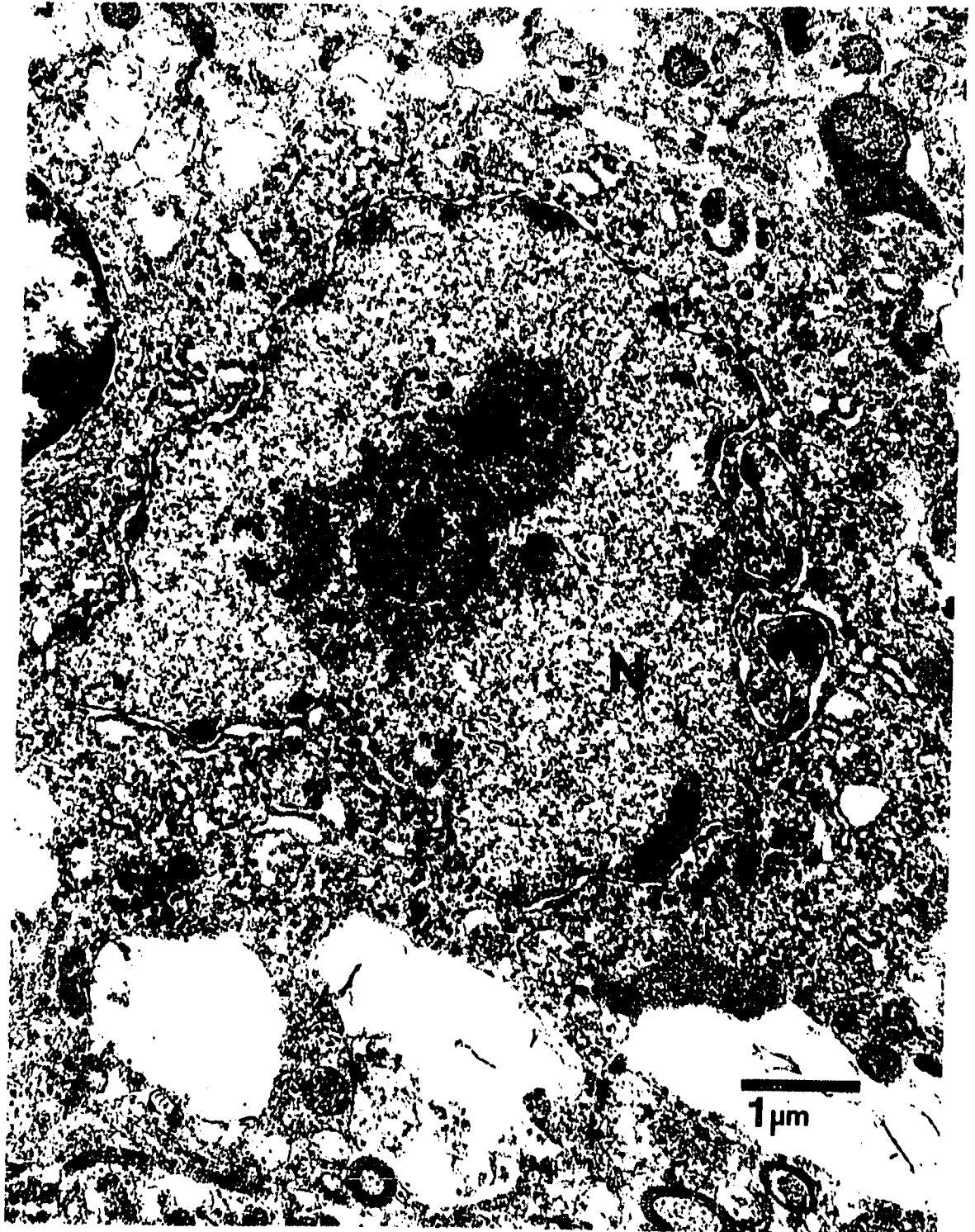




Figure 19. Dorsocaudal medulla, Cat 83, 7 DPI, Experiment I--Part B. Greater magnification of viral matrix from Figure 18 with small clusters and scattered nucleocapsids in the karyoplasm. Note incomplete (hollow) nonenveloped nucleocapsids (upper arrow) and complete (dark-staining core of nucleic acid) nonenveloped nucleocapsids (lower arrow). Uranyl acetate and lead citrate. x68,400

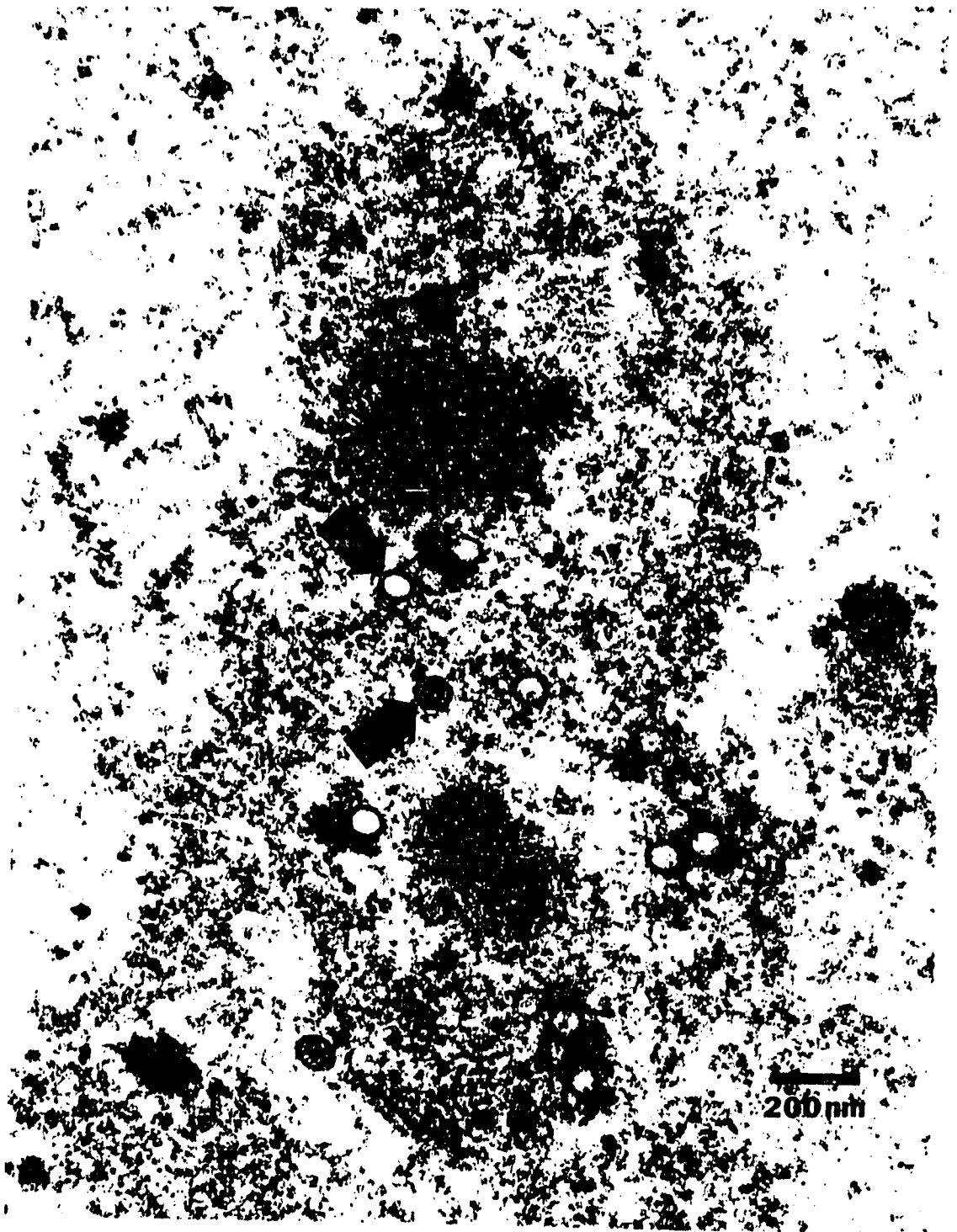


Table 5. Experiment II--Assignment of cats to experimental groups.

Group	Cat identification numbers			
I-Killed at initiation of clinical signs	56	61	64	67
II-Killed at more advanced stage of disease	41	69	70	71
III-Expired or killed at time near death	44	62	65	66

a manner similar to that described for Experiment I. Animals were examined visually twice daily for clinical signs of pseudorabies and were killed when showing clinical signs corresponding to the experimental group to which they had been assigned. Exaggerated, painful appearing attempts to swallow, anorexia and emesis were considered early clinical signs. Mild depression and withdrawal to the back of the cage, moderate salivation and a low, mournful growl, especially on expiration, were considered indicative of more advanced disease with the most advanced signs being marked salivation, continuous low growling sounds, loss of equilibrium and motor control of the limbs, and marked depression progressing to a comatose state and death. Postmortem examinations were performed on all cats and appropriate tissues were collected for light microscopy. Control cats from Experiment I--Part A served

as controls for histopathologic evaluation of tissues. No virus isolation studies were performed on tissues from these 12 animals.

Light microscopy      The pons, medulla, trigeminal ganglion, pharyngeal mucosa, tonsil, and tongue were collected, fixed and processed in a manner similar to tissues in Experiment I--Part A. The pons and medulla were sectioned transversely in their entirety. Two sections from each of 5 equally spaced areas of the pons and 5 equally spaced areas of the medulla were stained with H&E and LFB-CV. All other tissues were stained with H&E only.

### Results

Macroscopic lesions      The 12 cats were generally free of gross lesions except for Cat 69 from the 2nd group in which the leptomeninges covering the cerebral hemispheres were markedly edematous and covered diffusely with a yellow, finely granular exudate which caused flattened gyri and widened sulci. The latter were filled with gelatinous fluid. Similar meningeal changes of a lesser severity were found over the cerebellum and brain stem.

### Microscopic lesions

Tonsils      The histologic changes within the tonsils were similar in nature and severity in all 3 groups of animals. Focal to diffuse ulceration of crypt epithelium with degenerative, vacuolar changes in the epithelium adjacent to the ulcers were present. Macrophages and PMN's were present in varying numbers in the perifollicular, interstitial tissues and were especially numerous near ulcerated areas and within the crypt lumina.

Pharyngeal mucosa and tongue      These tissues were free of lesions.

Pons and medulla      The lesions present were of a type similar to those described for Experiment I--Part A. The severity of lesions varied from mild to marked in all 3 groups, however, the overall distribution of the lesions was noticeably different, especially between Group 1 and Group 3. In the 1st group, lymphocytic cuffing and microgliosis were limited mostly to the area of the nucleus and tractus solitarius and area postrema (Figures 20 and 21) and to the tissues along the course of the dorsal branches of cranial nerves 9 and 10 (Figures 22, 23, 24, and 25). Lesions were present in these areas in the 3rd group as well (Figures 26 and 27), however, focal and diffuse microglial proliferation and more marked lymphocytic cuffing of vessels were present throughout the parenchyma. Lymphocytic cuffing was moderate to marked around many of the major blood vessels along the median plane in the caudal medulla. The medullary lesions were least marked in Cat 71 from Group 2. In this cat, small, scattered microglial nodules were present along the tractus solitarius extending caudally to the commissural portion of the nucleus solitarius (Figure 28). Very slight lymphocytic cuffing of scattered parenchymal vessels was the only other lesion present in this animal. A multifocal to diffuse nonsuppurative leptomeningitis of the medulla was present in 2 cats from Group 2 and 2 from Group 3. Lymphocytes and macrophages were present within the pia mater and around subarachnoid vessels. In

Figure 20. Medulla, Cat 64, Group 1, Experiment II. Marked focal microgliosis of the area postrema (left side) and more diffuse microgliosis above and medial to the tractus solitarius (right side). H & E. x33

Figure 21. Medulla, Cat 64, Group 1, Experiment II. Greater magnification of right area postrema from Figure 20. Note mononuclear cuffing of vessels and small abscess within area postrema. H & E. x330

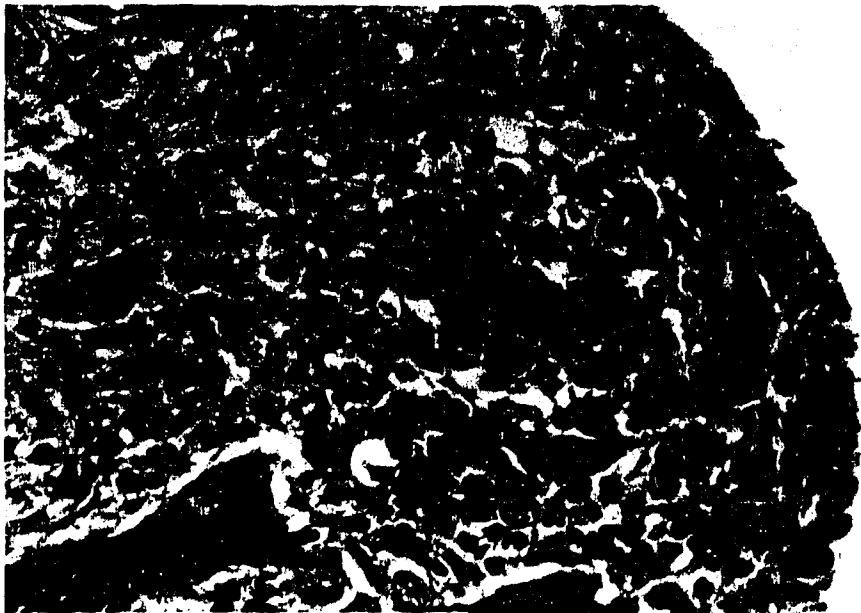
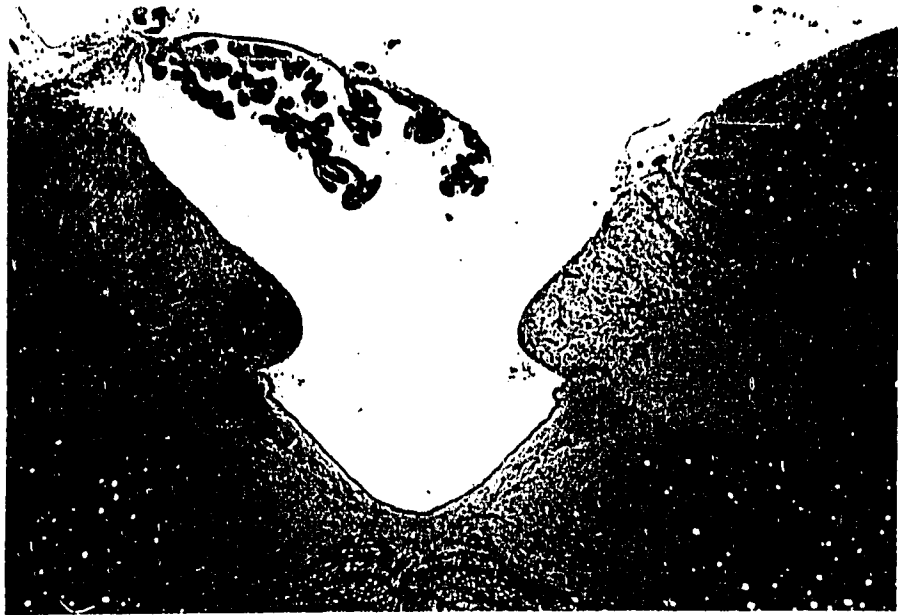


Figure 22. Medulla, Cat 56, Group 1, Experiment II. Focal microgliosis along the pathway of the 9th and 10th cranial nerves as they pass through the descending spinal tract (D) and nucleus (N) of the trigeminal nerve. H & E. x33

Figure 23. Medulla, Cat 56, Group 1, Experiment II. Greater magnification of portion of Figure 22. Note 2 distinct microglial nodules and small focus of microglial infiltration near lateral surface of medulla at the point of entrance of cranial nerves 9 and 10 into the medulla. H & E. x83



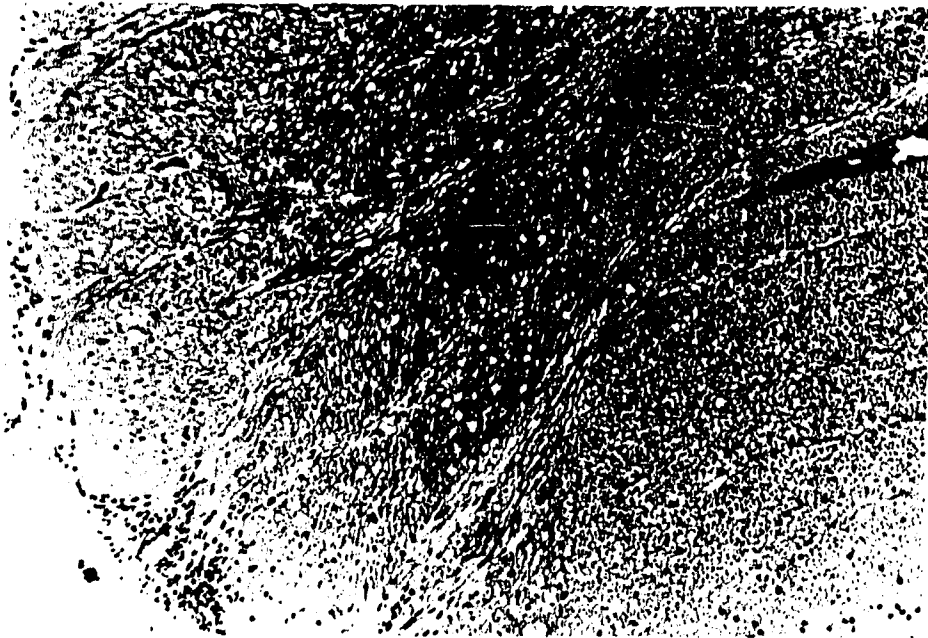
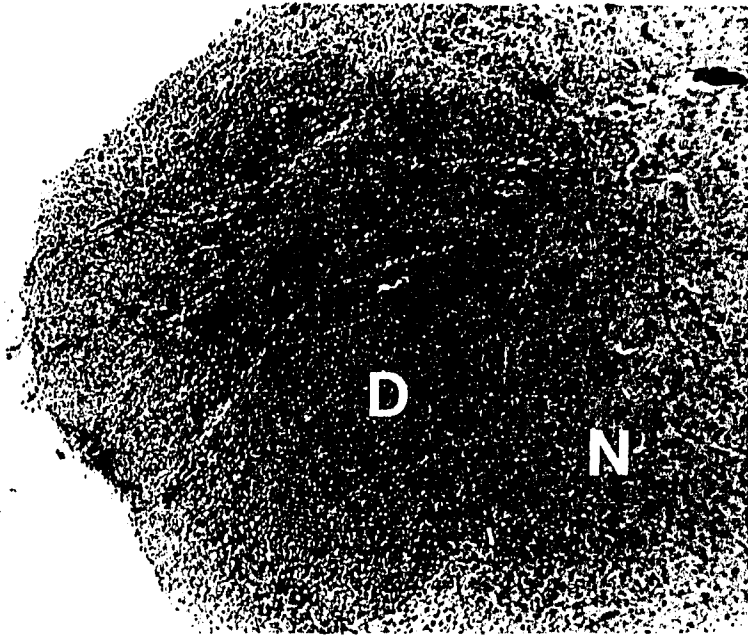


Figure 24. Medulla, Cat 56, Group I, Experiment II. Marked focal microgliosis (arrow) at point of entrance of 9th and 10th cranial nerves into the medulla. Note dilated, congested vessel above the descending spinal tract (D) and nucleus (N) of the trigeminal nerve. H & E. x33

Figure 25. Medulla, Cat 56, Group I, Experiment II. Greater magnification of the focal microgliosis from Figure 24. A few of the inflammatory cells are PMN's. H & E. x 165

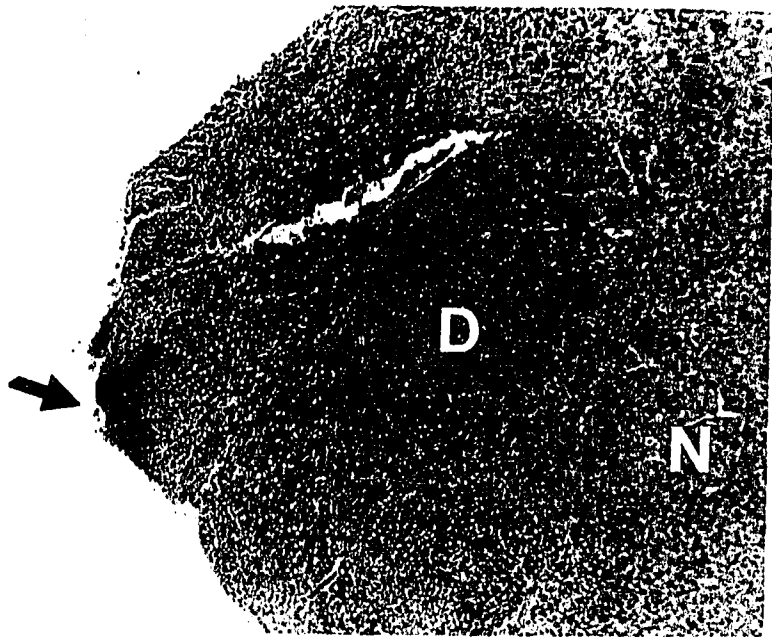


Figure 26. Medulla, Cat 66, Group 3, Experiment II. Marked focal microgliosis (arrow) of left side of medulla involving the area postrema (A) and nucleus solitarius. H & E. x53

Figure 27. Medulla, Cat 62, Group 3, Experiment II. Moderate microgliosis and lymphocytic perivascular cuffing at the point of entrance of the 9th and 10th cranial nerves and along their pathway over the descending spinal tract (D) of the trigeminal nerve. H & E. x33

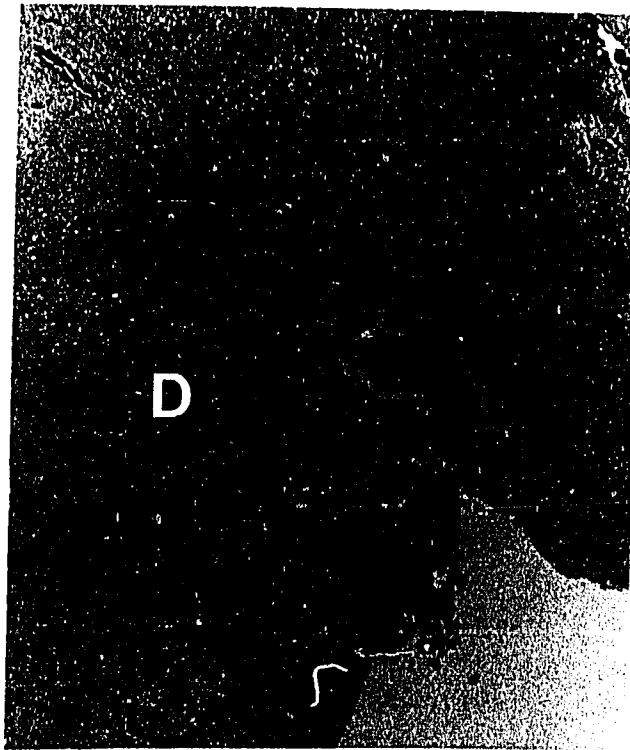
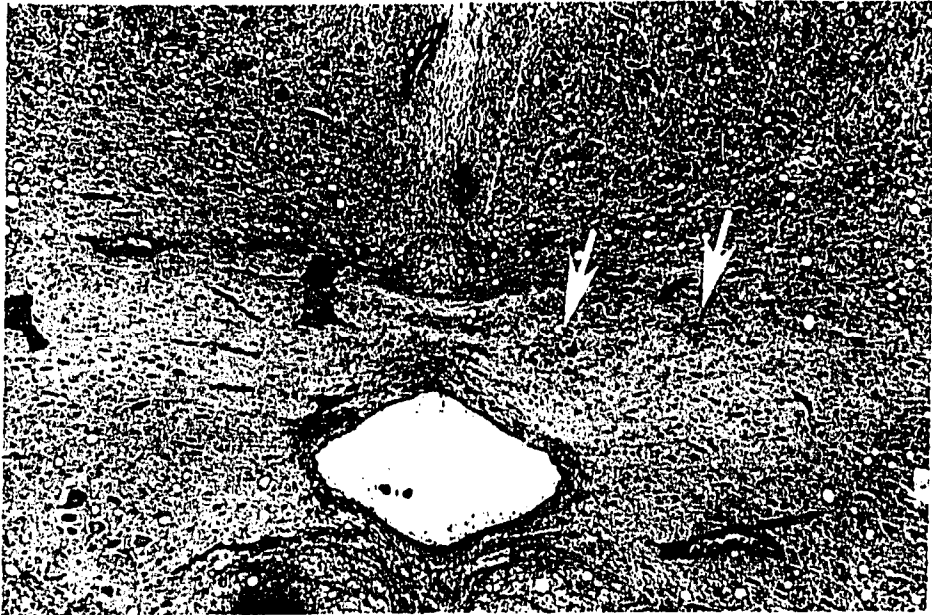


Figure 28. Medulla, Cat 71, Group 2, Experiment II. Mild multifocal microgliosis of commissural portion of the nucleus solitarius (right side-arrows). H & E. x53



1 cat from Group 2 (69), a cuff of histiocytes was present around several subarachnoid vessels as well as a mild vasculitis with necrosis of the tunica intima, medialis and adventitialis. The degree of cuffing, vasculitis and necrosis was considerably more marked in this cat than in any other animal. In Cat 70, a mild, focal leptomeningitis of the medulla was present at the site of entrance of the dorsal branches of cranial nerves 9 and 10.

Lesions in all cats were generally bilateral in distribution (Figure 29) though occasionally more marked unilaterally (Figure 30). The extent and severity of the bilateral nature of the lesions have been evaluated subjectively and reported in Table 6.

Trigeminal ganglion      Lesions were found in 2 of 4 cats from Group 1, 3 of 4 from Group 2, and 4 of 4 from Group 3, and generally consisted of 1 to 3 randomly scattered foci of microgliosis and lymphocytic infiltration of the interstitium with occasional foci of coagulation necrosis of the interstitium and infiltration of a few PMN's. Necrosis of a few scattered ganglion cell bodies was found, however, no intranuclear inclusions were identified. Several large necrotic foci heavily infiltrated with PMN's and macrophages were present in Cat 69. The lesions in this cat were much more suppurative and necrotic than those found in other cats in Experiments I or II.



Figure 29. Medulla, Cat 56, Group 1, Experiment II. Bilateral distribution of microglial nodules in dorsocaudal medulla. H & E. x33

Figure 30. Medulla, Cat 66, Group 3, Experiment II. Microgliosis (arrow) in dorsocaudal medulla with a predominantly unilateral distribution. H & E. x13

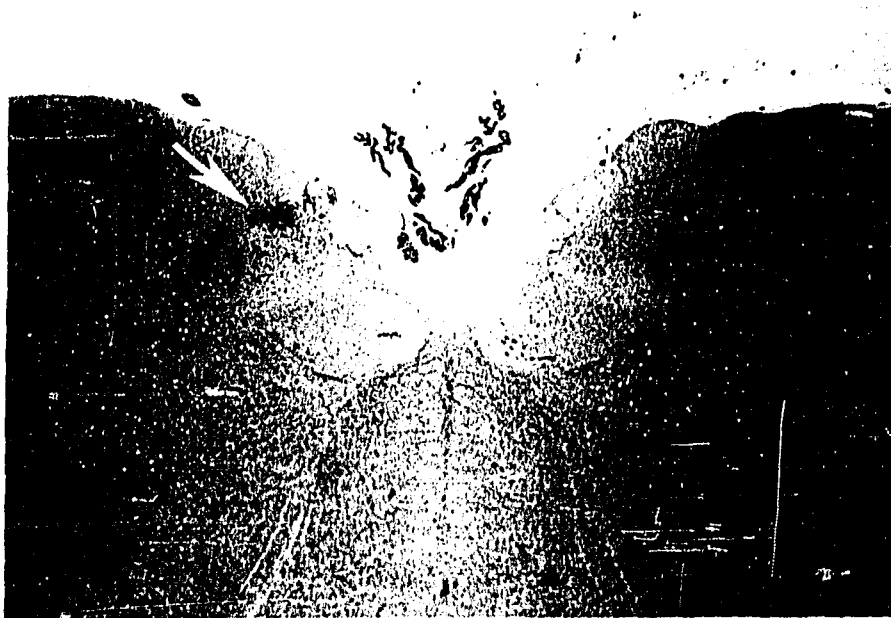


Table 6. Experiment II--Severity of microscopic lesions of selected tissues and bilateral distribution and severity of brain stem lesions.

Cat Number	I				Group II				III			
	56	61	64	67	41	69	70	71	44	62	65	66
Medulla	+++ <sup>a</sup>	++ <sup>b</sup>	+++	+++	++	++++ <sup>c</sup>	+++	+ <sup>d</sup>	++++	+++	+++	+++
Pons	+	++	- <sup>e</sup>	+	+	++++	++	-	++	++	++	-
Trigeminal ganglion	-	++	++	-	-	++++	+	+	+	+	++	++
Tonsil	+	++	+++	++++	+++	+++	++	++++	++	+++	+++	++
Bilateral distribution and severity of brain stem lesions	+++ <sup>f</sup> /+++	++ <sup>f</sup> /++	+ <sup>f</sup> /+++	+++ <sup>f</sup> /+++	++ <sup>f</sup> /++	++++ <sup>f</sup> /++++	+++ <sup>f</sup> /+++	+ <sup>f</sup> /+	++++ <sup>f</sup> /++++	+++ <sup>f</sup> /++	+++ <sup>f</sup> /+++	+++ <sup>f</sup> /+++

<sup>a</sup> Moderately severe lesions.

<sup>b</sup> Moderate lesions.

<sup>c</sup> Severe lesions.

<sup>d</sup> Mild lesions.

<sup>e</sup> No lesions present.

<sup>f</sup> Severity of brain stem lesions: on left / on right .

## Experiment III

Material and methods

Six cats, Numbers 58, 63, 72, 73, 74 and 75, were inoculated orally with 1 ml of a 1:10 dilution of stock virus in PBS. The oral and nasal mucosa were swabbed daily starting 1 DPI and continuing until the time of death. Swabs were placed in 1 ml of EBSS and stored at -70 C until inoculation onto PK15 cells. Specimens were considered negative if CPE was not found after 2 blind passages.

Results (Table 7)

Oral and nasal swabs yielded virus from all cats on the first day following inoculation. On the 2nd day, oral and nasal swabs yielded virus in all except 1 cat (75) in which both oral and nasal swabs were negative. Oral and nasal swabs were positive in 1 cat (58) and oral swabs were positive in 2 cats (74 and 63) on the 3rd DPI. All swabs were negative on the 4th day and remained negative until the respective time of death for each cat. Once an oral or nasal swab from a cat was negative, swabs obtained from the respective sites at later times remained negative. The time from inoculation until death among the 6 cats ranged from 4 to 7 days with a mean of 5.5 days.

Table 7. Experiment III--Virus isolation results from swabs of oral and nasal secretions collected daily following oral inoculation of pseudorabies virus.

Cat Number	Specimen	Days post inoculation							
		1	2	3	4	5	6	7	8
58	oral	+	+	+	-	-	-	N <sup>a</sup>	N
	nasal	+	+	+	-	-	-	N	N
63	oral	+	+	+	-	-	-	N	N
	nasal	+	+	-	-	-	-	N	N
72	oral	+	+	-	-	-	N	N	N
	nasal	+	+	-	-	-	N	N	N
73	oral	+	+	-	-	N	N	N	N
	nasal	+	+	-	-	N	N	N	N
74	oral	+	+	+	-	-	N	N	N
	nasal	+	+	-	-	-	N	N	N
75	oral	+	-	-	-	-	-	-	N
	nasal	+	-	-	-	-	-	-	N

<sup>a</sup> Cat died prior to obtaining daily swabs.

## DISCUSSION

The oral route of inoculation was chosen to approximate the situation on swine farms where cats are believed to be infected by ingesting dead, PRV-infected piglets. The quantity of virus in the inoculum was chosen somewhat arbitrarily based on pilot studies which demonstrated that cats are susceptible to PRV over a wide inoculum range. The manifestations of the disease produced by the selected inoculum resembled the disease in the field both with regard to clinical signs and duration.

### Clinical Signs

The development of clinical signs as described in Experiment II was very similar to that reported by Sabo et al. (1968). Exaggerated swallowing movements, vomition and excessive salivation early in the course of the disease were consistent with histologic lesions found in the nucleus and tractus solitarius and area postrema, areas closely related morphologically to the centers of deglutition, emesis, and salivation (Morest, 1967). Exaggerated swallowing movements may also have been enhanced by pain associated with the ulcerative, purulent tonsillar cryptitis present at 2 DPI and later. The more advanced central nervous system (CNS) signs such as loss of equilibrium and depression which progressed to a comatose state correlated well with the more diffuse, severe CNS lesions described in cats killed at 4 and 5 DPI in Experiment I--Part A and in cats killed in the terminal stages of the disease in Experiment II.

In the present study, like that of Sabo et al. (1968), pruritus was consistently absent. In both studies, virus was inoculated without the presence of bones or other orally-abrasive agents. Metianu et al. (1971) were unable to reproduce pseudorabies in their cats until the inoculum was mixed with bones or other abrasive materials. Pruritus was present in their cats and Horvath and Papp (1967) observed pruritus in 60 percent of the field cases on which they reported. This suggests that abrasive materials, which are usually present in pig carcasses, may damage mucous membranes and thus permit entrance of virus to the tissues of the gingiva and lips. Passage of virus via the trigeminal nerve would then be enhanced, damage to the trigeminal ganglia and brain centers would be likely, and pruritus of the face would be an expected sequellum. Since pruritus was not present in any cats in the present study, no comparisons could be done between the presence or lack of pruritus and the histopathologic changes in the trigeminal nerve centers of the CNS. Correlative histopathologic studies of field cases in which pruritus has or has not been recorded should be done to better evaluate the reasons for the presence or absence of pruritus. In those cats in which pruritus has been reported, other signs such as salivation, emesis, retching, and depression were also observed. This suggests that the pathogenic mechanisms of psuedorabies in cats proposed by this study are basically the same, with or without pruritus. A possible, though less plausible reason, in the author's opinion, for the presence or absence of pruritus would be inherent differences in the virus isolates studied.

### Macroscopic Lesions

Pulmonary lesions were present in both control and inoculated cats from Experiment I--Part A. The virological and bacteriological histories of these cats were unknown. However, clinical signs of respiratory disease were not observed in these animals during the course of the experiment. Therefore the gross and microscopic lesions of the lung were considered incidental to the experimental disease. The absence of gross lesions specific for pseudorabies in other systems agrees with the findings of Sabo et al. (1968). In the dog and mink, diphtheritic enteritis, ulceration of the esophagus and stomach, and severe gastritis have been reported by Gore et al. (1977) and Christodoulou et al. (1970), respectively, suggesting an altered pathogenesis in the dog and mink as compared with the cat. Gross lesions in other carnivores such as the fox and ferret are unusual except for self-inflicted focal areas of subcutaneous edema.

### Microscopic Lesions of Nonnervous Tissue

The ulcerative, tonsillar cryptitis suggests a probable route of entry of the virus and is compatible with the findings of Sabo et al. (1968), who found viral antigen in the squamous epithelium of the tonsils and the surrounding pharyngeal mucous membrane using fluorescent antibody-histoimmunologic techniques. Fluorescent antibody techniques were not employed in the present study, so the specific location of viral antigen was not determined. Sabo et al. (1968) observed a positive fluorescence in lymphocytes and reticular cells and in the sensory nerve fibers in the perifollicular tonsillar tissue on the 4th and 5th days after



inoculation. In the present study, virus was isolated from the medial retropharyngeal lymph node of only 2 cats, both of which were killed 5 DPI. This finding suggests that virus enters regional lymphatic channels only after several days of replication in epithelial or nervous tissue. The later appearance of virus in the regional lymphatics suggests that dissemination of the virus via the lymphatic channels and eventually by the blood stream is not an important consideration in the pathogenesis since clinical signs and histologic lesions of the nervous system were generally well-developed by the 3rd and 4th DPI.

#### Microscopic Lesions of Nervous Tissue

The most significant finding to suggest the primary route by which the virus enters the medulla was found in the medulla and pons of the cats from Experiment I--Part A killed 3 DPI and later. The earliest lesions detectable were generally located in or around the tractus and nucleus solitarius and along the pathway of the sensory branches of the glossopharyngeal and vagus nerves. Foci of intense gliosis were often found at the point where these nerves emerge from the lateral border of the medulla. The tractus solitarius is composed of sensory fibers from the facial, glossopharyngeal and vagus nerves which supply sensory innervation, including taste, to the tongue and pharynx. The nucleus solitarius lies mostly medial to the tract and the right and left nuclei join to form the commissural portion of the nucleus solitarius in the extreme caudodorsal medulla just above and caudal to the obex. The earliest and most severe lesions were consistently found in this area of the brain stem and were generally found at times which correlated

well with the clinical signs and virus isolation results. Two exceptions were found in the cats killed 2 DPI and before. The medulla, pons and midbrain from Cat 38 killed 1 DPI yielded virus though no lesions were found histologically. The early presence of virus in the brain and the absence of microscopic lesions is difficult to explain. One possibility may have been the presence of an ulcer or similar undetected lesion of the mouth or pharynx which might have allowed rapid viral penetration of mucous membranes and access to nerve endings thus facilitating rapid transport of virus to the brain. Likewise, the presence of lesions in the cerebellum, cerebral hemispheres, brain stem and all spinal cord segments in Cat 25 killed 2 DPI was unusual. Only salivary gland, pharyngeal mucosa-tonsils, and cervical spinal cord yielded virus. Possibly a viremia of short duration may have spread the virus throughout the nervous system with enough virus present to stimulate an inflammatory response and too little virus to isolate by the methods used in this experiment.

Moderate to severe lesions of the central nervous system, especially the medulla and pons, were found in all 18 cats killed 3 DPI or longer. Of these, however, nervous tissue failed to yield virus in 6 cats. Only the cervical spinal cord of 1 cat killed 2 DPI yielded virus though 3 of 7 cats killed 2 DPI had microscopic lesions of the CNS. It is clear that under the conditions of this experiment, the presence of microscopic lesions of the nervous system was a more consistent though less specific indicator of PRV infection than was virus isolation. Virus was found in the absence of lesions only in Cat 38 killed 1 DPI. A degree of

variability between lesions and virus isolation results is expected because of the experimental design, since the brain was hemisected longitudinally, one-half being examined for virus and the other half for histology. This made it impossible to examine the same tissue for both virus and lesions. Cat 62 from Experiment II is an example of one in which lesions were mild on one side and moderately severe on the other (Table 6).

The specific area of the brain stem in which lesions were most consistently found in the present study is not in agreement with the findings of either Sabo et al. (1968) or Dow and McFerran (1963). The latter investigators reported lesions from a single cat to be located particularly in the main sensory and spinal tract nuclei of the trigeminal nerve. Typical inclusions were reported in many neurons of these nuclei. Sabo et al. (1968) reported CNS lesions most consistently in the vestibular nuclei, dorsal nucleus of the vagus nerve, the nucleus of origin of the facial nerve and the reticular formation. None of these nuclei are directly related to the trigeminal nuclei. However, his work concludes that the primary route of the virus from the pharynx to the brain is via the trigeminal nerve.

Microscopic lesions were found in the trigeminal ganglion of 4 of 7 cats killed 4 DPI or later from Experiment I--Part A and of 8 of 11 cats examined from Experiment II. However, lesions in the pons or medulla directly related anatomically to the trigeminal ganglion were not found while lesions in areas directly related to the tractus and nucleus solitarius were found in virtually all cats having any lesions

of the CNS. These results are conclusive that under the conditions of these experiments, the nerves associated with the tractus solitarius, principally the sensory branches of the glossopharyngeal and vagal nerves are much more important viral pathways from the pharynx to the medulla than is the trigeminal nerve.

The meningeal lesions described in several cats in Experiment II may not have resulted entirely from PRV infection. In Cat 69 especially, the degree of necrotic vasculitis and the granulomatous inflammation of the meninges, as well as the multiple pyogranulomatous foci found in the trigeminal ganglion, are highly compatible with the nonexudative form of feline infectious peritonitis (FIP) (Slauson and Finn, 1972). This form of FIP may have a long course without clinical signs of disease and the lesions described are nearly identical to those found in Cat 69. The meningitis found in several other cats in Experiment II was much milder, nonsuppurative, and generally more limited to the basilar portions of the brain stem. This reaction appeared much more likely to extend from the more intense focal meningitis often found at the point of entrance of the glossopharyngeal and vagus nerves from the lateral margin of the medulla (Figures 22 and 24). This milder lymphocytic leptomeningitis was also reported by Sabo et al. (1968).

#### Ultrastructural Findings

Based upon the proposed pathways of herpes viruses to the CNS and the known sites of viral replication within the nucleus of infected cells, the presence of herpes nucleocapsids within neurons of the medulla was to be expected. The failure to demonstrate herpes-like inclusion

bodies by light microscopy in sections of brain and ganglia indicated that the number of nucleocapsids concentrated within the nuclei of affected cells would be small. Using light microscopy, affected neurons could be identified only by the margination of chromatin and the pale, lytic appearance of the central portion of the nucleus. Ultrastructurally, seldom were more than 10 to 12 nucleocapsids found within a nucleus and at no time were large aggregations of virus present. Focal areas of necrosis with an intense infiltration of acute inflammatory cells were not characteristic findings in the medulla at the light level and this too suggested that the concentration of viruses or viral proteins would not be great.

Unfortunately, ultrastructural studies did not reveal viruses within the epithelial cells of the tonsillar crypts. At the light microscopic level, the presence of focal areas of ulceration and degenerative changes such as vacuolation of the epithelial cells adjacent to ulcerated foci are all strong evidence for the epithelial cells being initial sites of viral replication. Intranuclear inclusions within tonsillar crypt epithelial cells were not found by light microscopy in any of the tonsils examined which also indicated that the concentration of viruses within these cells would not be large. The failure to find viruses in epithelial cells at the ultrastructural level is therefore not considered strong evidence against some viruses being present in damaged epithelial cells.

The results of virus isolation studies indicate that infective virus is generally present in both the pharyngeal mucosa-tonsillar tissues and in the medulla of cats inoculated 3 days or later even

though it is difficult to demonstrate viral particles in these infected tissues ultrastructurally. This suggests that the development of additional techniques such as fluorescein labeled antibody tests or immunoperoxidase staining of infected tissues is desirable if the precise location of virions or viral proteins within infected tissues is to be determined.

#### Virus Isolations from Oral and Nasal Secretions

The results of virus isolation attempts from oral and nasal swabs following oral inoculation are in marked contrast to similar studies by Sabo et al. (1968). In the present study, swabs from oral and nasal secretions yielded virus for the first 2 DPI, and sometimes the 3rd DPI, while specimens collected 4 DPI or later were consistently negative. In contrast, Sabo et al. (1968) isolated virus from the saliva only after the 3rd DPI. As in the present study, they also were unable to isolate virus from the salivary gland of cats with virus in the saliva and concluded that the source of virus in saliva was from sloughed, infected cells of the pharyngeal wall and tonsils. The pharyngeal mucosa and tonsils in the present study consistently yielded virus, however, an increase in virus titer in this tissue from the 3rd to 5th DPI, similar to that found by Sabo et al. (1968), could not be ascertained because no attempt was made to determine virus titers in tissues. Dow and McFerran (1963) were also unable to isolate virus from the saliva of a naturally-infected cat in which the pharyngeal mucosa yielded virus. The source of the virus isolated from the saliva for the first 3 days in the present study was believed to be residual virus from the inoculum. No reasons

are offered to explain the failure of Sabo et al. (1968) to isolate residual virus from saliva for the 1st 2 days since the inoculum in the present study and that of Sabo et al. (1968) were comparable, being approximately  $10^6$  pfu in 1 ml PBS and  $10^6$  pfu in 5 ml of milk, respectively. Differences between the results of virus isolation attempts from oral secretions could be expected if the effective dosage of virus in the present study was significantly greater than that of Sabo et al. (1968). Two observations suggest that this may be true. First, clinical signs were observed regularly on the 3rd DPI while they were not observed until the 4th day in the study by Sabo et al. (1968). Also, microscopic lesions in the brain stem were not found until 5 DPI in the former study while they were observed in several cats killed 2 DPI and consistently found in cats killed 3 DPI and later in the present experiment. Additional variability in results of virus isolation attempts from oral secretions and tissues and in lesions produced may have resulted from differences in virus strains used in these experiments.

The results of virus isolation attempts from oral secretions in the present study indicate that only a small amount of virus, if any, is present in saliva during the mid to terminal stages of clinical disease when excessive salivation is a prominent clinical sign. This suggests that sick, salivating cats do not spread significant amounts of virus from farm to farm. Virus, however, is generally present in the tonsils and brains of cats dead of pseudorabies and access to these carcasses by PRV-susceptible swine represents a potential hazard.

## SUMMARY AND CONCLUSIONS

Domestic cats were inoculated orally with an Iowa isolate of pseudorabies virus. Several cats were killed at intervals of 1 day and examined virologically and histologically to determine the initial sites of virus penetration and replication and to evaluate the pathways traveled by the virus from the mouth to the central nervous system. Consistent lesions in the tonsils, the nucleus and tractus solitarius and the area postrema of the caudal medulla, and along the course of the glossopharyngeal and vagus nerves suggest that the virus enters the body through the mucous membranes of the tonsil, replicates in the tonsillar epithelium and surrounding interstitial tissues and passes to the brain stem along the sensory branches of the glossopharyngeal and vagus nerves. Less consistent lesions in the trigeminal ganglion and nuclei of the trigeminal nerve indicate a lesser role for this nerve pathway in the pathogenesis of pseudorabies in the cat. Generally, virus isolations correlated well with the microscopic lesions. With one isolated exception, no evidence for a general hematogenous or lymphatic dissemination of virus in the initial course of the disease was found. Some suggestion for dissemination of virus to the regional lymphatics of the head, later in the course of the disease, was found.

Ultrastructurally, herpesviruses were observed within the nucleus of neurons of the medulla but not in selected ganglia of cranial nerves or in the pharyngeal mucosa-tonsillar tissues even though parallel



virus isolation studies suggested the presence of virus in these tissues. Alternate techniques such as fluorescein labeled antibody or immunoperoxidase staining methods are suggested as possible techniques to demonstrate the precise location of virions or viral proteins within infected tissues.

Clinical signs consisted of anorexia, listlessness, exaggerated swallowing movements, salivation, emesis, low-pitched growling sounds, depression, coma, and death. Pruritus was consistently absent. At the initiation of the earliest clinical signs, lesions were bilaterally distributed in the brain stem, though occasionally more marked unilaterally. As the disease progressed clinically, the extent and severity of lesions increased throughout the parenchyma of the brain stem. Lesions of nervous tissue generally consisted of multifocal to diffuse microgliosis, mononuclear vascular cuffing and a mononuclear inflammatory cell infiltration of the above mentioned areas with a variable infiltration of neutrophils occasionally resulting in microabscess formation. Lesions were characterized by neither intranuclear inclusion bodies nor specific neuronal changes.

Attempts to isolate virus from oral and nasal swabs were consistently unsuccessful 4 days or longer after inoculation even though parallel studies indicated the virus was present in the tonsil and pharyngeal mucosa at that time. This suggests that pseudorabies infected cats play a minor role if any in the dissemination of virus while they are salivating during the mid to terminal stages of the disease. However, the pharyngeal

mucosa and tonsils probably contain viable virus until death and longer and represent a hazard to susceptible animals if cats dead from pseudo-rabies are ingested.

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